Oxytocin
A new treatment for anorexia nervosa?

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Oxytocin – a new treatment for anorexia nervosa?

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Abstract

The course of anorexia nervosa (AN) is protracted and it remains a serious illness with the highest mortality rate among psychiatric disorders. Risk and maintenance models of AN suggest that difficulties in social-emotional processes and elevated stress and threat sensitivity play an important role in the development of AN and interact with the core processes further fuelling the illness. This thesis explores the question whether oxytocin is useful candidate to enhance treatment for anorexia nervosa. Chapter 2 systematically reviews studies examining the impact of experimentally induced negative and positive mood on eating behaviour across eating and weight disorders. The meta-analyses demonstrated that negative mood has an impact on eating behaviour particularly among people with subclinical and clinically-relevant difficulties around food and eating. Studies 2 and 3 investigate the neural processes that underlie anomalies in implicit social-emotional processing in AN. The functional neuroimaging findings showed that during implicit processing of facial affect people with AN have anomalies on neurofunctional level, in regions including the amygdala, prefrontal cortex, and insula. Finally, studies 4 – 7 explore a potential new treatment, the neuropeptide oxytocin, and examine its effect on threat-related processing and social-emotional difficulties. The findings demonstrated that although intranasal oxytocin may modulate some aspects of threat processing, such as cortisol response and attentional bias among people with AN, it has little effect on social-emotional processing. Taken together, the findings indicate that there is currently little evidence to support the use of intranasal oxytocin in the treatment of AN or other psychiatric disorders. Interestingly, these findings also suggest that subjective mood can have a strong impact on eating behaviour and show that people with AN have anomalies in neural activation during implicit processing of social-emotional cues. New treatments targeting these anomalies may be helpful in the treatment of AN.

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List of abbreviations

AB	Attentional bias
ACTH	Adrenocorticotropic hormone
AN	Anorexia nervosa
AQ-10	Autism Spectrum Quotient (short version)
ASD	Autism spectrum disorder
BED	Binge eating disorder
BMI	Body mass index
BN	Bulimia nervosa
BOLD	Blood oxygen level dependent
BPD	Borderline personality disorder
CBT	Cognitive behavioural therapy
CBT-ED	Cognitive behavioural therapy for eating disorders
CI	Confidence interval
CNS	Centre for Neuroimaging Sciences
CRF	Corticotropin releasing factor
CSF	Cerebrospinal fluid
DARTEL	Diffeomorphic Anatomical Registration using Exponentiated Lie algebra
DASS	Depression Anxiety Stress Scales
DLPFC	Dorsolateral prefrontal cortex
DSM	Diagnostic and statistical manual for mental disorders
ED(s)	Eating disorder(s)
EDEQ	Eating Disorder Examination Questionnaire
EEG	Electroencephalogram
EMA	Ecological momentary assessment

EMG	Electromyography
EPI	Echo planar imaging
ES	Effect size
F	Female
FACES	Facial Expression Coding System
FDR	False discovery rate
fMRI	Functional magnetic resonance imaging
FTD	Frontotemporal dementia
FWHM	Full width at half maximum
GABA	Gamma-Aminobutyric acid
GMI	Guided mood induction
GRADE	Grading of Recommendations Assessment, Development and Evaluation
GWAS	Genome-wide association study
h	Hours
HC(s)	Healthy control(s)/comparison(s)
HEDT	Hexagon emotion discrimination task
HPA	Hypothalamic–pituitary–adrenal
HRF	Hemodynamic response function
IAPS	International Affective Picture System
IPT	interpersonal therapy
IRLS	Iteratively re-weighted least squares
ITI	Inter-trial interval
IU	International unit
Kcal	Kilocalories
M	Male

MANTRA	Maudsley Anorexia Nervosa Treatment for Adults
MEG	Magnetoencephalography
min	Minutes
ml	Millilitres
MNI	Montreal Neurological Institute
MP-RAGE	Magnetization-prepared rapid gradient echo
MRI	Magnetic resonance imaging
ms	Milliseconds
MSCEIT	Mayer-Salovey Caruso Emotional Intelligence Test
N	Sample size
NHS	National health service
NICE	National Institute for Health and Clinical Excellence
NIHR	National Institute for Health Research
NR	Not reported
NRES	National Research Ethics Service
OCD	Obsessive-compulsive disorder
OED	Others' emotional display
PANAS	Positive and Negative Affect Schedule
PFC	Prefrontal cortex
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PTSD	Post-traumatic stress disorder
Q1	1st quartile
Q3	3rd quartile
RCT	Randomised controlled trial
RE(s)	Restraint eater(s)

RMET	Reading the mind in the eyes
ROI	Region of interest
s	Seconds
SD	standard deviation
SE	Standard error
SF	Social feedback
SMC	Standardised mean change
SPM	Statistical Parametric Mapping
SSCM	Specialist supportive clinical management
SSRI(s)	Selective serotonin reuptake inhibitor(s)
sub-BED	sub-clinical binge eating disorder
TASIT	The Awareness of Social Inference Test
TE	Echo time
TR	Repetition time
VAS	Visual analogue scale
VLPFC	Ventrolateral prefrontal cortex
WFU	Wake Forest University
WHO	World health organisation

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Statement of work

Chapter 1: Introduction

The candidate's own work that was shaped with constructive comments from her supervisors Prof Janet Treasure and Dr Kate Tchanturia.

Chapter 2:

The candidate conducted the literature search and screening for eligibility along with Dr Valentina Cardi. The candidate conducted the meta-analyses and contributed to interpretation of the findings and drafting of the final manuscript along with all co-authors.

Chapters 3 and 4:

The candidate contributed to data collection along with Dr Valentina Cardi and Mirko Esposito. The pre-processing and subject level analysis of the neuroimaging data were conducted by Dr Owen O'Daly, and the candidate conducted second level, group analysis. The statistical analysis of the behavioural and demographic data was the candidate's own work. Interpretation of the findings was the candidate's own work with help from her supervisors Prof Janet Treasure and Dr Kate Tchanturia as well as the other co-authors.

Chapters 5 and 6:

The data collection was the candidate's own work with help from Dr. Kah Wee Ng and Sara Moss. The saliva samples were analysed by the Via Path. The candidate performed all statistical analyses and Dr

John Hodsoll provided statistical consultation. Interpretation of the findings was the candidate's own work with help from her supervisors Prof Janet Treasure and Dr Kate Tchanturia as well as the other co-authors.

Chapters 7 and 8:

The literature search and screening for eligibility was the candidate's own work with help from Dr Kah Wee Ng. The candidate conducted the meta-analyses and Dr John Hodsoll provided statistical consultation. Interpretation of the findings was the candidate's own work with help from her supervisors Prof Janet Treasure and Dr Kate Tchanturia as well as the other co-authors.

Chapter 9: Discussion

Candidate's own work that was shaped with constructive comments from her supervisors Prof Janet Treasure and Dr Kate Tchanturia.

Publications

List of publications features in this thesis

Cardi, V., **Leppanen, J.**, & Treasure, J. (2015). The effects of negative and positive mood induction on eating behaviour: A meta-analysis of laboratory studies in the healthy population and eating and weight disorders. Neuroscience and Biobehavioral Review, 57, 299-309. doi:10.1016/j.neubiorev.2015.08.011

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Leppanen, J., Cardi, V., Ng, K. W., Paloyelis, Y., Stein, D., Tchanturia, K., & Treasure, J. (2017b). The effects of intranasal oxytocin on smoothie intake, cortisol and attentional bias in anorexia nervosa. Psychoneuroendocrinology, 79, 167-174. doi:10.1016/j.psyneuen.2017.01.017

Leppanen, J., Cardi, V., Ng, K. W., Paloyelis, Y., Stein, D., Tchanturia, K., & Treasure, J. (2017a). Effects of intranasal oxytocin on interpretation and expression of emotions in anorexia nervosa. Journal of Neuroendocrinology, 31, 31. doi:https://dx.doi.org/10.1111/jne.12458

Leppanen, J., Ng, K. W., Kim Y. R., Tchanturia, K., & Treasure, J. (2018). Meta-analytic review of the effects of a single dose of intranasal oxytocin on threat processing in humans. Journal of Affective Disorders, 225, pp 167-179 doi:10.1016/j.jad.2017.08.041

Leppanen, J., Ng, K. W., Tchanturia, K., & Treasure, J. (2017). Meta-analysis of the effects of intranasal oxytocin on interpretation and expression of emotions. Neuroscience and Biobehavioral Review, 78, 125-144. doi:10.1016/j.neubiorev.2017.04.010

List of other publications completed during the PhD but not incorporated in this thesis

Tchanturia, K., Giombini, L., **Leppanen, J.**, Kinnaird, E. (2017). Evidence for Cognitive Remediation Therapy in Young People with Anorexia Nervosa: Systematic Review and Meta-analysis of the Literature. European Eating Disorders Review, 25 (4), pp. 227-236. DOI: 10.1002/erv.2522

Leppanen, J., Dapelo, M.M., Davies, H., Lang, K., Treasure, J., Tchanturia, K. (2017). Computerised analysis of facial emotion expression in eating disorders. PLoS One, 12 (6). DOI: 10.1371/journal.pone.0178972

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Davies, H., Wolz, I., **Leppanen, J.**, Fernandez-Aranda, F., Schmidt, U., Tchanturia, K. (2016). Facial expression to emotional stimuli in non-psychotic disorders: A systematic review and meta-analysis. Neuroscience and Biobehavioral Reviews, 64, pp. 252-271. DOI: 10.1016/j.neubiorev.2016.02.015

Westwood, H., Eisler, I., Mandy, W., **Leppanen, J.**, Treasure, J., Tchanturia, K. (2016). Using the Autism-Spectrum Quotient to Measure Autistic Traits in Anorexia Nervosa: A Systematic Review and Meta-Analysis. Journal of Autism and Developmental Disorders, 46 (3), pp. 964-977. DOI: 10.1007/s10803-015-2641-0

Treasure, J., Cardi, V., **Leppanen, J.**, Turton, R. (2015). New treatment approaches for severe and enduring eating disorders. Physiology and Behavior, 152, pp. 456-465. DOI: 10.1016/j.physbeh.2015.06.007

Cardi, V., Corfield, F., **Leppanen, J.**, Rhind, C., Deriziotis, S., Hadjimichalis, A., Hibbs, R., Micali, N., Treasure, J. (2015). Emotional processing, recognition, empathy and evoked facial expression in eating

disorders: An experimental study to map deficits in social cognition. PLoS One, 10 (8). DOI: 10.1371/journal.pone.0133827

Hibbs, R., Rhind, C., **Leppanen, J.**, Treasure, J. (2015). Interventions for caregivers of someone with an eating disorder: A meta-analysis. International Journal of Eating Disorders, 48 (4), pp. 349-361. DOI: 10.1002/eat.22298

Outline of thesis

Chapter 1 provides a general introduction to anorexia nervosa. The general introduction will provide an overview of aetiology of anorexia nervosa (AN), including diagnostic criteria, risk factors, and maintaining factors. The introduction will also explore the possible role of the neuropeptide oxytocin in the risk and maintaining processes underlying anorexia nervosa. **Figure 1** shows an outline of the thesis structure.

Chapter 2 consists of a published meta-analytic review paper examining the effects of experimentally induced positive and negative mood on eating behaviour across eating and weight disorders.

Chapter 3 consists of a published neuroimaging paper investigating neural processes that underlie difficulties in implicit processing of facial affect in AN.

Chapter 4 consists of a published neuroimaging paper, which builds on the previous study examining the neural correlated of processing of implicit infant emotion in AN.

Chapter 5 consists of a published experimental research paper exploring the effects of a single dose of intranasal oxytocin on anxiety, physiological stress, and threat-related attentional processing of food stimuli in AN.

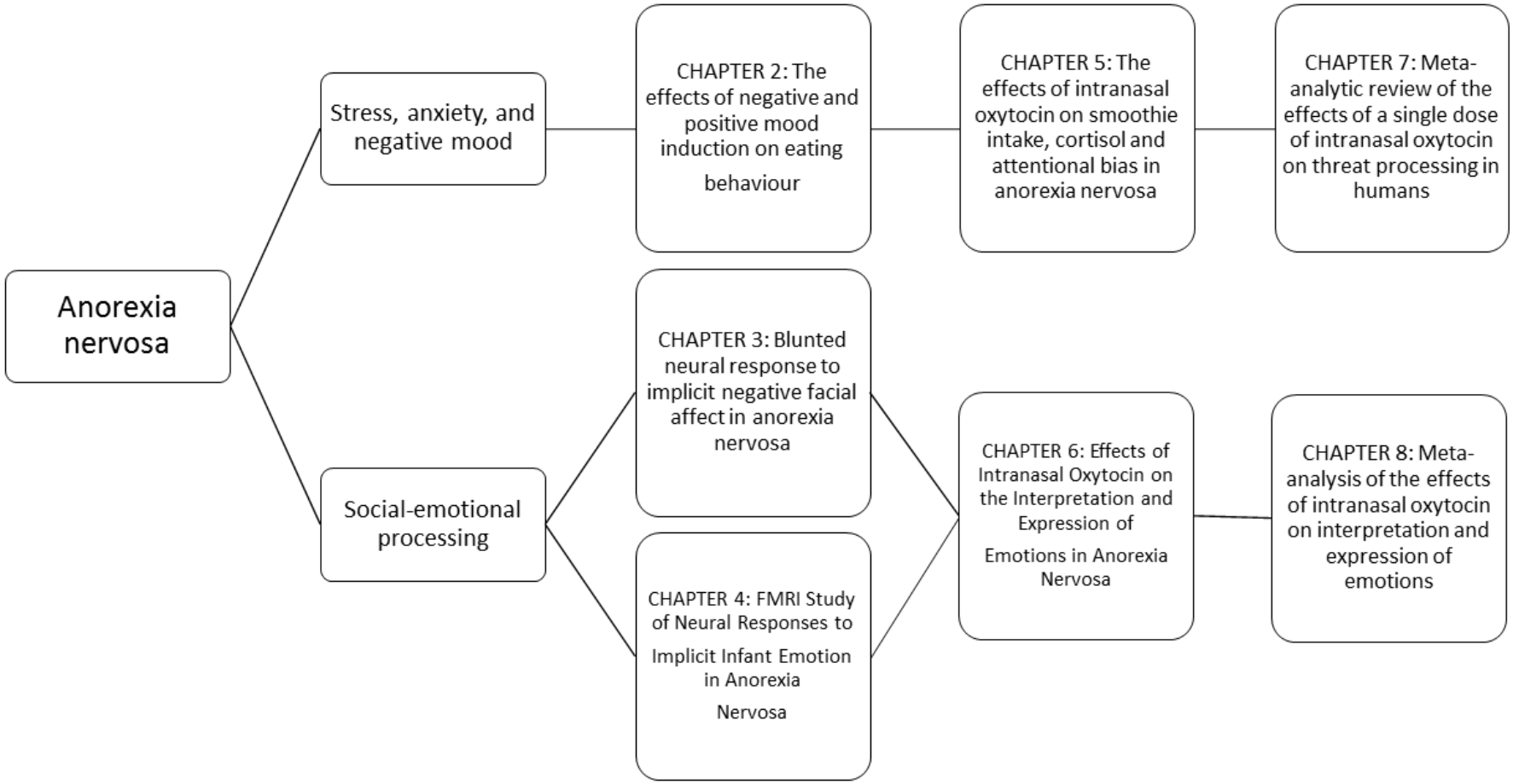
Chapter 6 consists of a published experimental research paper exploring the effects of a single dose of intranasal oxytocin on interpretation and expression of emotions in AN.

Chapter 7 consists of a meta-analytic review paper investigating the effects a single dose of intranasal oxytocin on various aspects of threat processing across healthy and clinical populations. The paper is currently under review after minor revisions.

Chapter 8 consists of a published meta-analytic review paper investigating the effects a single dose of intranasal oxytocin on interpretation and expression of emotions across healthy and clinical populations.

Chapter 9 provided general discussion of findings, limitations, and conclusions.

Figure 1. Outline of the thesis structure



CHAPTER 1:

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1 Introduction

1.1 Anorexia nervosa

1.1.1 Diagnostic criteria

The fifth edition of the Diagnostic and Statistical Manual for Mental Disorders (DSM-5 (American Psychiatric Association, 2013)) states the following criteria for diagnosis of anorexia nervosa (AN):

Table 1. DSM-5 diagnostic criteria for anorexia nervosa

Criterion	Description
A	Significantly low caloric intake relative to requirements and significantly low body weight relative to age, sex, physical health, and developmental trajectory
B	Severe fear of weight gain or becoming fat and/or persistently engaging in behaviours that leads to weight loss
C	Disturbances in perception of body shape and weight, strong relationship between body image and self-esteem or sense of self-worth

To meet Criterion A an individual is required to maintain low weight along with refusal to gain weight, which can be defined in a variety of ways. According to the World Health Organisation (WHO, 1995), body mass index (BMI) of 17 kg/m^2 or below indicates moderate to severe thinness, while BMI below 16 is a marker of serious risk to health (WHO, 1995). However, the DSM-5 recommends that clinicians consider additional information including clinical history, body build, and physiological health in addition to weight for height indicators to support the diagnosis.

Criterion B postulates that people with AN exhibit intense fear of weight gain and becoming fat that is not attenuated by weight loss. Instead, weight loss often intensifies these fears and increases desire to engage in behaviours that lead to further weight loss, which is likely fuelled by disturbances in the perception of body shape and weight described in Criterion C. These disturbances are also closely connected to the individual's self-esteem whereby weight loss is seen as an achievement and a sign of self-control, whereas weight gain is seen as a failure and a sign of loss of control.

In addition to these three criteria the DSM-5 lists some additional factors that clinicians may use to support of supplement diagnosis. These include low mood or depression as well as social-emotional difficulties, including reduced facial communication and social isolation.

1.1.2 Epidemiology

AN is a complex mental disorder that typically develops during adolescence and is more prevalent among females than males (Lucas et al., 1991; Favaro et al., 2009; Raevuori et al., 2014). Recent large-scale community cohort studies have estimated the prevalence of AN to be between 1.7 and 4.2 % among young females (Smink et al., 2012; Smink et al., 2014; Mustelin et al., 2016). In community cohort studies, the annual incidence rate of broad AN among young females has been estimated to be 270 – 1204 in 100 000 (Keski-Rahkonen et al., 2007; Smink et al., 2012). Studies examining medical records report lower annual incidence rates of 4.2 – 109.2 in 100 000, suggesting many patients do not present for treatment (Smink et al., 2012; Keski-Rahkonen and Mustelin, 2016; Smink et al., 2016).

1.1.3 Heritability

Research suggests that AN is highly heritable with family studies demonstrating that first-degree relatives have relative risk of 11.3 to develop the illness relative to the general population (Strober et al., 2000; Brandys et al., 2015). Additionally, twin studies have documented that heritability index, h^2, between 0.56 (95% CI 0.00, 0.87) and 0.76 (95% CI 0.35, 0.95) (Klump et al., 2001; Bulik et al., 2006). However, twin studies have been criticised for a number of drawbacks, including inflating results due to reduced non-shared environment between the twins (Fairburn et al., 1999; Klump et al., 2009). However, adoption studies, which by-pass some of these criticisms, support findings from twin studies and demonstrated substantial biological heritability of 59%-82%, with the remaining variance being explained by unique environmental factors (Klump et al., 2009).

1.2 Current treatments in AN

In May 2017 the National Institute of Health and Care Excellence (NICE) updated the 2004 guidelines for the treatment of eating disorders (NICE, 2017). At present, there are no gold standard treatments for AN according to NICE primarily due to lack of research evidence. There is currently low to very low GRADE evidence for the use of psychological and psychopharmacological interventions in the treatment of adults with AN. Furthermore, there is a substantial lack of well conducted randomised controlled trials (RCTs) with adequate statistical power and follow-up assessments to support any treatment recommendations (NICE, 2017).

1.2.1 Psychological therapies

The goal of psychological therapy has been suggested to be to reduce risks and increase motivation to gain and maintain healthy weight, and promote both physical and psychological recovery (NICE, 2017). The NICE guidelines recommend psychological therapies, including cognitive behavioural therapy (CBT-ED), Maudsley Anorexia Nervosa Treatment for Adults (MANTRA), and specialist supportive clinical management (SSCM), to be considered in the treatment of AN (NICE, 2017). However, the evidence base for these interventions is still small. Although, recent small RCTs suggest that CBT-ED may be helpful in targeting key eating disorder cognitions and increase BMI in AN (Fairburn et al., 2013; Fairburn et al., 2015), replication of these findings in larger trials remains necessary before firm recommendations can be made. Only two RCTs investigating the effectiveness of MANTRA have thus far been conducted and both trials indicate that the intervention may be effective in increasing BMI and reducing core eating disorder psychopathology, but improvements were not significantly greater than those seen in patients in the control condition (Schmidt et al., 2012; Schmidt et al., 2015). Finally, there is some emerging evidence that SSCM may be helpful in targeting low mood and general functioning in AN, but there is still little evidence that SSCM produces

significantly greater improvement over and above other interventions (Carter et al., 2011; Watson and Bulik, 2012; Touyz et al., 2013).

1.2.2 Psychopharmacological therapies

The NICE guidelines recommend that psychopharmacological therapies should not be considered as first line treatments in AN, but should be considered to compliment psychological therapy (NICE, 2017). The goals of psychopharmacological therapy should be to promote weight gain and target comorbid symptoms such as depression and anxiety (NICE, 2017). At present, the evidence suggests that antidepressants are unlikely to promote weight gain or improve psychopathology in AN (Hay and Claudino, 2012; Zipfel et al., 2015). Thus, there has been increasing interest in exploring the potential of antipsychotics, such as olanzapine, in targeting maladaptive cognitions and anxiety during weight restoration in AN (Brambilla et al., 2007; Treasure et al., 2010; Hay and Claudino, 2012). However, pharmacological therapies in the treatment of AN are not without risks, including a potential increase in the QTc interval and associated elevated risk of cardiac arrhythmia (Treasure et al., 2010; NICE, 2017). Therefore, recently the effectiveness of alternative pharmacological interventions with fewer side-effects utilising synthetic forms of neuropeptides, such as oxytocin, have been explored in the treatment of AN (Maguire et al., 2013).

1.2.3 Summary of current treatments in AN

There are currently no gold standard treatments for AN (NICE, 2017). The importance of further research into the causal and maintaining mechanisms in eating disorders as well as the mechanisms that could support change has been highlighted (Jansen, 2001, 2016). These findings could be used to guide treatment and inform the development of new, more effective interventions (Jansen, 2001, 2016). Recently steps have been taken towards incorporating research evidence into the development

of psychological interventions such as MANTRA (Schmidt et al., 2012; Schmidt et al., 2013; Schmidt et al., 2015). However, many of the maintaining factors that are automatic in their nature are difficult to target through top-down therapies (Treasure and Schmidt, 2013); and currently available pharmacological treatments have failed to show robust evidence of being able to promote weight gain and effectively target psychopathology in AN (Treasure et al., 2010; Zipfel et al., 2015). Thus, there is growing need for better understanding the mechanisms that underlie AN and exploring the potential of new, pharmacological agents, such as neuropeptides, and neurotechnology in the treatment of the disorder (Schmidt and Campbell, 2013; Treasure et al., 2015b).

1.3 RDoC – a new way to conceptualise psychiatric disorders

The Research Domain Criteria (RDoC), spearheaded by the United States National Institute of Mental Health, has been introduced as a new way to conceptualise psychiatric disorders in order to improve understanding and help guide treatments. The RDoC is a set of dimensional psychological constructs developed to conceptualise human behaviour and psychiatric disorders (https://www.nimh.nih.gov/research-priorities/rdoc/index.shtml). It is divided into five main domains that describe emotional, cognitive, and motivational functioning in humans and are presented in **Table 2**.

Table 2. Research Domain Criteria

Domain	Description	Methods of analysis
Negative valence system	The negative valence systems are primarily involved in supporting functions such as threat processing, aversive emotional states, and anxiety and stress responses. These systems are involved in activation of the body's fight or flight systems, including the hypothalamic-pituitary-adrenal axis, and are activated in the face of threat. However, these systems may also become hypersensitive or overactive even when no clear threat is present.	These systems can be investigated in experimental research by examining physiological startle response, attentional bias, and stress hormone levels.

Domain	Description	Methods of analysis
Positive valence system	The positive valence systems are primarily involved in supporting reward learning, reward processing, and motivational responses. The system is activated when a person is exposed to learned cues that trigger motivated approach responses or willingness to work in order to gain a reward. Under some certain circumstances these systems can give rise to maladaptive behaviours.	These systems can be investigated in experimental research by examining impulsivity, compulsive behaviours, and repetitive behaviours.
Cognitive systems	The cognitive systems are involved in supporting a number of cognitive processes, including attention, working memory and language. Under some circumstances these processes can become maladaptive, leading to cognitive rigidity and anomalies in memory bias.	These systems can be investigated in experimental research by examining divided and undivided attention, working memory capacity, and cognitive flexibility.
Systems of social processes	The systems of social processes include a variety of functions involved in interpersonal interaction, such as attachment, theory of mind, and interpretation and expression of facial affect. In some case anomalies in these processes may rise causing interpersonal difficulties.	These systems can be investigated in experimental research by examining attachment formation and maintenance, facial expressions, and understanding of self and others.

Domain	Description	Methods of analysis
Arousal/regulatory systems	The arousal and regulatory systems support functions such as homeostatic balance, including energy maintenance and sleep/wake cycles. In some cases, these processes can become dysregulated leading to insomnia and hyper- or hypoarousal.	These systems can be investigated in experimental research by examining normal, daily sleep and eating behaviour, and sleep cycles.

Although each domain is of relevance to AN (Wildes and Marcus, 2015), more information is provided below on two of the domains most pertinent to this thesis: the negative valence system and the social processes domains.

1.3.1 Negative valence system domain in AN

Current maintenance models of AN postulate that increased stress and threat sensitivity in AN may play a role in the development and progression of core eating disorder behaviours (Connan et al., 2003; Strober, 2004). Elevated stress appears apparent on physiological level in AN with several studies reporting high levels of peripheral stress hormone, cortisol and CRF (Gold et al., 1986; Licinio et al., 1996; Monteleone et al., 2011; Oskis et al., 2012). High cortisol and CRF availability in AN has been suggested to be due to increased production, which indicates that there may be hyperactivation of the hypothalamic pituitary adrenal (HPA) axis (Walsh et al., 1978). This hypothesis is supported by exploration of the adrenocorticotropic hormone (ACTH) response to a CRF challenge in people with AN, which points towards hyperactivation of the HPA and anomalies in the hypothalamic, negative

feedback loops (Gold et al., 1986). Taken together these findings suggest that the hyperactivated HPA axis may be an important target for treatments in AN.

Elevated anxiety, stress, and negative mood play an important role in the maintenance of AN, fuelling core eating disorder behaviours, such as restriction and food avoidance (Connan et al., 2003; Kaye et al., 2009). Studies using daily momentary assessment to track mood and behaviour have reported that in AN dietary restriction and food avoidance are often preceded by episodes of low mood, stressful life events, and high anxiety (Engel et al., 2013; Lavender et al., 2013). These findings suggest that core eating disorder behaviours may be compensatory behaviours used to control anxiety, stress, and negative mood (Haynos and Fruzzetti, 2011; Brockmeyer et al., 2012). However, malnutrition in turn leads to increases in dysphoric mood and anxiety, over time creating a vicious cycle (Connan et al., 2003; Kaye et al., 2009).

Chapter 2 in this thesis further explores the impact of experimentally manipulated mood and stress on eating behaviour across eating and weight disorders.

As the illness progresses food and eating become increasingly associated with stress and anxiety, and pathoplastic changes in information processing can develop (Williamson et al., 1999; Schmidt and Treasure, 2006; Yackobovitch-Gavan et al., 2009; Steinglass et al., 2011; Monteleone et al., 2017). Experimental work has shown that people with AN show elevated negative facial reactions and report increased fear and disgust in response to food stimuli (Léonard et al., 1998; Uher et al., 2004; Soussignan et al., 2011). Further work has also reported anomalies in attentional biases towards food and eating related stimuli in AN, suggesting that people with AN may view these stimuli as threatening (Brooks et al., 2011; Aspen et al., 2013; Werthmann et al., 2015). Together these findings point towards neuroprogressive nature of AN such that elevated illness-related threat sensitivity increases

fear and stress around food and eating, thus leading to increased reliance of safety behaviours, such as food avoidance (Steinglass et al., 2011; Treasure et al., 2015a). Thus, threat sensitivity and stress fuelled reactions to illness-related stimuli may play an important role in the maintenance of AN and may be important targets for interventions (Steinglass et al., 2011; Heeren et al., 2015).

1.3.2 Systems of social processes domain in AN

A recent series of meta-analyses have reported the people with AN show range of anomalies in social-emotional processing, including high rates of insecure early attachment and significant difficulties in the accurate interpretation of complex emotions and emotional theory of mind (Oldershaw et al., 2011; Caglar-Nazali et al., 2014; Bora and Kose, 2016). These difficulties may contribute to the interpretation biases and anomalies in reactivity to social-emotional cues that have also been reported in AN (Ambwani et al., 2015; Dapelo et al., 2016; Cardi et al., 2017). Relative to healthy individuals, people with AN perceive salient, emotional stimuli to be colder and more negative (Cardi et al., 2014b; Ambwani et al., 2015). Furthermore, people with AN display fewer positive and negative facial expressions while viewing emotionally provoking stimuli (Davies et al., 2016). Together, these findings suggest that people with AN may have specific difficulties in implicit processing and reactivity to social-emotional cues.

Chapter 3 of this thesis further explores the neural processes that underlie anomalies in implicit processing of facial affect in people with AN.

Anomalies in social-emotional processing of stimuli depicting other adults may be confounded by elevated sensitivity to social rank and implicit threat making it difficult to determine whether these anomalies are present due to difficulties in processing the emotional cues or rank sensitivity (Troop and Baker, 2008; Cardi et al., 2013; Cardi et al., 2014a; Troop, 2016). However, a recent study by Cardi

et al. (2014b) investigated differences in the interpretation and expression of emotions in response to infant emotional displays, which were used to elicit caregiving responses. People with eating disorders expressed less positive facial affect and made fewer positive interpretations while viewing positively valenced infant stimuli (Cardi et al., 2014b). Additionally, people with eating disorders reported more subjective negative affect in response to negatively valenced infant stimuli (Cardi et al., 2014b). These findings suggest that social-emotional processing anomalies in AN may extend across the lifespan.

Chapter 4 of this thesis further explores the neural processes that underlie anomalies in implicit processing of infant emotional display in people with AN.

Difficulties in interpretation of others' emotions contribute to impairments in social communication leading to difficulties in forming and maintaining relationships (Gross, 2002; Hooker and Park, 2002; Fett et al., 2011; Kothari et al., 2013; Tchanturia et al., 2013). Indeed, people with AN report fewer friendships and greater isolation (Levine, 2012; Doris et al., 2014; Westwood et al., 2016). Increased isolation or perceived isolation, in turn, has been found to impair mental and physical health, and elevate social threat sensitivity (Cornwell and Waite, 2009; Cacioppo et al., 2010; Cacioppo and Cacioppo, 2014). Isolation is also believed to give more space to eating disorder thoughts, negatively impact brain functioning, and perpetuate reliance on core eating disorder behaviours as coping mechanisms (Fairburn et al., 2003; Treasure and Schmidt, 2013; Treasure et al., 2015a). Thus, taken together, these findings indicate that difficulties in social-emotional processing may be important targets for treatments in AN.

1.4 Oxytocin

The endogenous neuropeptide, oxytocin, is primarily produced in the hypothalamus (Onaka, 2004). It acts centrally in several regions within the brain and is released into the periphery via the pituitary gland (Onaka, 2004). It has been implicated in several distinct functions, including stress and threat processing, and social functioning, and has been suggested to be a potential new target for interventions in psychiatry. In the section below we summarise the preclinical and clinical studies, which have been used to inform the potential role of oxytocin as a form of treatment.

1.4.1 Findings from preclinical research

Preclinical studies have documented that oxytocin plays an important role in the stress response to potential threats in an animal's environment (Onaka, 2004; Yoshida et al., 2009; Onaka et al., 2012). Psychological and physical stress give rise to the physiological stress and anxiety response by activating the HPA axis and the amygdala leading to increased release and availability of glucocorticoids (Onaka, 2004; Gobrogge and Wang, 2015). Glucocorticoids, including corticotropin releasing factor (CRF), then feedback to the hypothalamus activating the oxytonergic neuron in the paraventricular nucleus of the hypothalamus, which in turn begin to release oxytocin (Onaka, 2004; Kormos and Gaszner, 2013; Gobrogge and Wang, 2015). Oxytocin is thought to play a role in a negative feedback loop, promoting the activity and release of an inhibitory neurotransmitters, such as GABA, which in turn suppress the increased activation in the HPA axis and the amygdala (Yoshida et al., 2009; Onaka et al., 2012; Gobrogge and Wang, 2015). Thus, oxytocin is thought to be a type of anxiolytic, keeping other stress and anxiety responses from overshooting (Yoshida et al., 2009; Onaka et al., 2012; Gobrogge and Wang, 2015).

Experimental preclinical studies have provided substantial support for this model, demonstrating that oxytocin release during stressful tasks appears to suppress HPA axis hyperactivation (Smith et al., 2016). Acute administration of exogenous oxytocin has also been found to reduce fear and threat reactivity and anxiety-like behaviours, such as attentional bias towards threat in monkeys and hiding in corners during an open field test in rodents (Yoshida et al., 2009; Parr et al., 2013; Neumann and Slattery, 2016). Additionally, a recent systematic review reported that if administered in the rodent amygdala or infralimbic cortex exogenous oxytocin reduced fear responses to learned threat, facilitating fear extinction (Neumann and Slattery, 2016). Furthermore, exogenous oxytocin administration has also been found to facilitate adaptation to chronic stress (Zheng et al., 2010; Olszewski et al., 2016). Experimental work has found that acute administration of exogenous oxytocin promotes normal functioning of the digestive organs during periods of heterotypic stress, including cold restraint and forced swimming (Zheng et al., 2010). Similarly, exogenous oxytocin has been found to normalise eating behaviour when exposed to anxiety-provoking novel environment (Olszewski et al., 2016). Taken together, these findings suggest that oxytocin has the potential to be an anxiolytic agent promoting normal functioning during periods of stress and anxiety.

Preclinical studies have also suggested that endogenous oxytocin is involved in social functioning, contributing to social recognition, pair bonding, and social interaction in animals (Onaka et al., 2012; Hurlemann and Scheele, 2016). The exact mechanisms through which oxytocin contributes to social functioning are currently unclear, but evidence thus far suggests that circuits within and connecting the hypothalamus, amygdala, and the striatum may be involved (Onaka et al., 2012). Oxytocin release from the hypothalamus promotes maternal behaviour while lesions in this region reduce oxytocin availability, and impair and delay the initiation of such behaviour (Lim and Young, 2006; Crockford et al., 2014; Hurlemann and Scheele, 2016). Additionally, lesions in the medial amygdala have been found to reduce oxytocin release and impair the quality of inter-male social interaction in mice (Onaka et al.,

2012; Wang et al., 2013). Furthermore, recent experimental work has demonstrated that social reward may be dependent on coordinated activity between oxytocinergic and serotonergic neurons in the mouse striatum (Dölen et al., 2013). Taken together, these findings suggest that oxytocin may be involved in promoting prosocial behaviour in animals.

1.4.2 Findings from human research

The above findings have sparked increasing interest to explore the role of oxytocin in humans. In humans, however, the relationship between oxytocin and the negative valence system appears to be more complicated than in rodents (Cardoso et al., 2014; Crockford et al., 2014). A recent meta-analytic review found that a single dose of intranasal oxytocin did not have a significant impact on cortisol response to laboratory stress tests among healthy individuals (Cardoso et al., 2014). Conversely, people with clinical disorders characterised by elevated anxiety and hyperactivation and dysregulation of the HPA axis, showed a significant, moderate-sized decrease in cortisol response to stress (Cardoso et al., 2014). Interestingly, anomalies in the levels of endogenous oxytocin and endogenous oxytocin response have also been identified among people with elevated anxiety and stress sensitivity (Crockford et al., 2014; Rutigliano et al., 2016). A recent meta-analytic review found that people with AN have significantly lower endogenous oxytocin levels than healthy individuals, which the authors suggested to be potentially related to the hyperactivation of the HPA axis (Rutigliano et al., 2016). Another experimental study reported anomalies in endogenous oxytocin response to stressful stimuli among women, who scored high on neuroticism, but not in women scoring low on neuroticism (Sanders et al., 1990). Taken together, these findings suggest that the oxytocin system may be a useful target to alleviate increased stress and anxiety in humans.

Recent experimental work has attempted to build on the above findings to explore whether oxytocin can also influence anxiety- and threat-related behaviours in humans (Bertsch et al., 2013; Domes et al., 2013b; Domes et al., 2013a; Kim et al., 2014a). Among healthy humans, oxytocin has been reported to reduce attention towards threat and increase attention towards positive stimuli (Domes et al., 2013b; Domes et al., 2013a). Similarly, among people with clinical disorders characterised by elevated threat sensitivity, oxytocin has been found to reduce attentional bias towards social threat (Bertsch et al., 2013; Domes et al., 2016). Moreover, a recent proof of concept pilot study reported that a single dose of intranasal oxytocin reduced attentional bias towards food, eating, and negative body shape related images in people with AN (Kim et al., 2014a). Taken together with the findings above, these findings suggest that oxytocin may be a potential treatment to target elevated anxiety- and threat-related behavioural responses in clinical populations.

More recently there has been interest in exploring the effects of intranasally delivered oxytocin on the systems of social processes in humans (Van Ijzendoorn and Bakermans-Kranenburg, 2012; Shahrestani et al., 2013). Previous meta-analytic reviews have suggested that intranasal oxytocin improves the recognition of basic emotions, and increases trust among healthy individuals with small effect sizes (Van Ijzendoorn and Bakermans-Kranenburg, 2012; Shahrestani et al., 2013). Additionally, experimental studies have documented that a single dose of intranasal oxytocin improves the interpretation of complex emotions and mental states among healthy individuals (Domes et al., 2007; Woolley et al., 2014; Feeser et al., 2015). Interestingly, previous behavioural work has also suggested that a single dose of oxytocin increases emotional empathy, congruent emotion expression, and social communication (Shamay-Tsoory et al., 2013; Korb et al., 2016; Spengler et al., 2016). Taken together, these findings suggest that oxytocin may be an important facilitator of pro-social behaviour.

Encouraged by findings among healthy individuals, there has been much interest in investigating the effects of intranasal oxytocin on social-emotional processing difficulties among clinical populations as well as its potential as a treatment enhancer (MacDonald and Feifel, 2013; Treasure et al., 2015b). A recent meta-analytic review reported small, but generally positive effect of intranasal oxytocin on social-emotional functioning and psychopathology among people with ASD, anxiety disorders, depression, schizophrenia, and borderline personality disorder (BPD) (Bakermans-Kranenburg and van Ijzendoorn, 2013). Additionally, experimental studies have found that a single dose of intranasal oxytocin improves accurate interpretation of complex emotions among people with schizophrenia and depression (MacDonald and Feifel, 2013; Woolley et al., 2014). A single dose of intranasal oxytocin has also been found to facilitate social communication and expression of emotions among people with schizophrenia and BPD (Brune et al., 2015; Woolley et al., 2017). Finally, recent pilot studies in eating disorders have found that a single dose of intranasal oxytocin attenuates attentional bias towards negative facial expressions in AN and improves sensitivity to recognise basic emotions in bulimia nervosa (Kim et al., 2014b; Kim et al., 2015). Thus, further exploration of the effects of intranasal oxytocin on social-emotional processing in AN may be of interest.

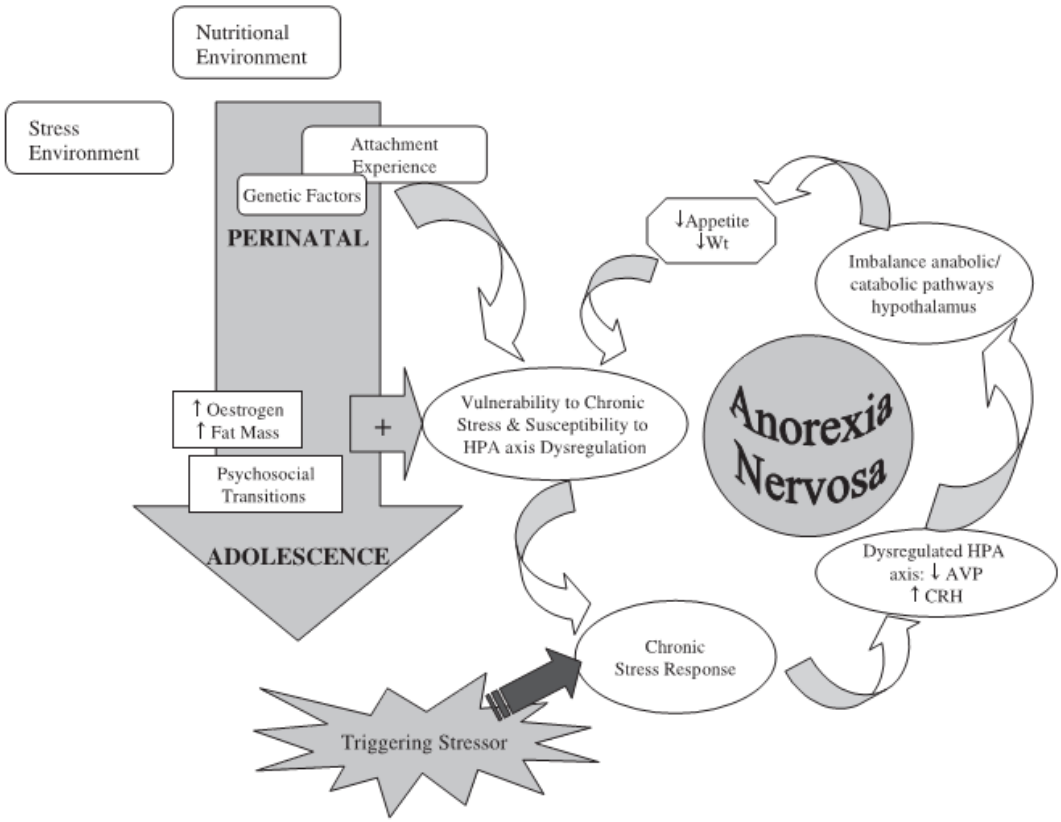
1.5 Maintenance models of anorexia nervosa

Theoretical models aim to deepen the understanding of the core eating disorder symptoms and risk factors, and explain how these processes contribute to the development and maintenance of disordered eating. There are currently dozens of models that describe the factors that contribute to the maintenance of anorexia nervosa (Pennesi and Wade, 2016). The models most pertinent for this thesis are described in more detail below along with suggestions of how the proposed maintaining factors could be modulated by oxytocin.

1.5.1 The neurodevelopmental model of anorexia nervosa (Connan et al., 2003)

The neurodevelopmental model of AN is presented in **Figure 2**. The model postulates that genetic factors along with attachment difficulties and anomalies in the negative valence system, including the physiological response to interpersonal stress, contribute to the development of AN (Connan et al., 2003). Attachment and other early life difficulties are believed to influence the development of neurobiological systems, such as the hypothalamic-pituitary-adrenal (HPA) axis, altering the development of associated feedback mechanisms. Preclinical studies have documented that together with genetic vulnerabilities, early life difficulties are associated with anomalies in the functional architecture of the HPA axis leading to chronically elevated level of glucocorticoid receptors and CRF (Holmes et al., 2005). Chronically elevated physiological stress is believed to reflect failures in adaptation to stress and in stress-induced up-regulation of inhibitory mechanisms and neuropeptides, such as oxytocin, and give rise to poor coping styles (de Kloet et al., 2005; Zheng et al., 2010). When an individual enters puberty, a time of considerable biological, psychological, and social challenges, these predisposing factors may contribute to susceptibility to AN (Connan et al., 2003). Once the illness is established the above changes along with the predisposing factors are likely to contribute to the maintenance of disordered eating giving a false sense of safety and control.

Figure 2. The neurodevelopmental model of anorexia nervosa (Connan et al., 2003)

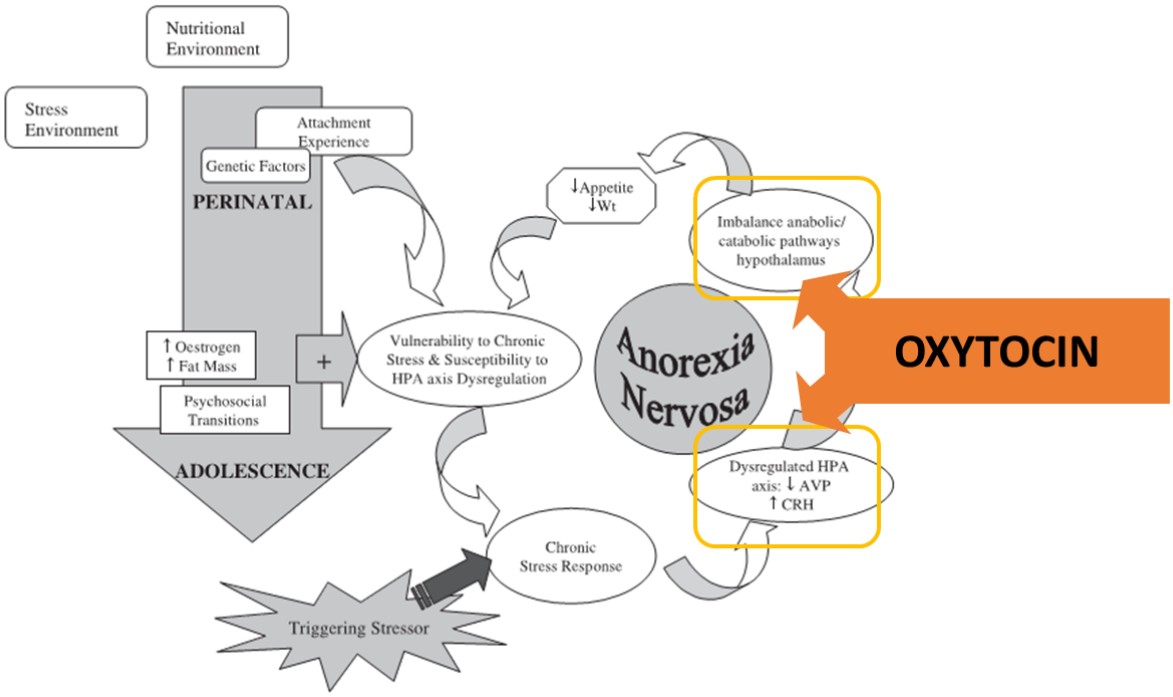


In the neurodevelopmental model of AN, the neuropeptide oxytocin could be beneficial in improving hyperactivation and dysregulation of the HPA axis and improve the functioning of inhibitory feedback mechanisms (**Figure 3**). A wealth of preclinical studies as well as recent experimental work among people with clinical disorders has demonstrated that oxytocin may be an effective anxiolytic (Yoshida et al., 2009; Onaka et al., 2012; Cardoso et al., 2014; Crockford et al., 2014; Gobrogge and Wang, 2015). Oxytocin is believed to reduce the stress-induced hyperactivation of the HPA axis by increasing the release and availability of inhibitory neurotransmitters such as GABA (Yoshida et al., 2009; Onaka et al., 2012; Gobrogge and Wang, 2015). Thus, in the neurodevelopmental model of AN, oxytocin may

improve core eating disorder symptoms by lowering the chronic state of neurobiological stress and reducing reliance on such maladaptive coping mechanisms.

Chapter 5 in this thesis explores the effects of a single dose of intranasal oxytocin on salivary cortisol in AN.

Figure 3. Potential site of oxytocin action in the neurodevelopmental model of AN

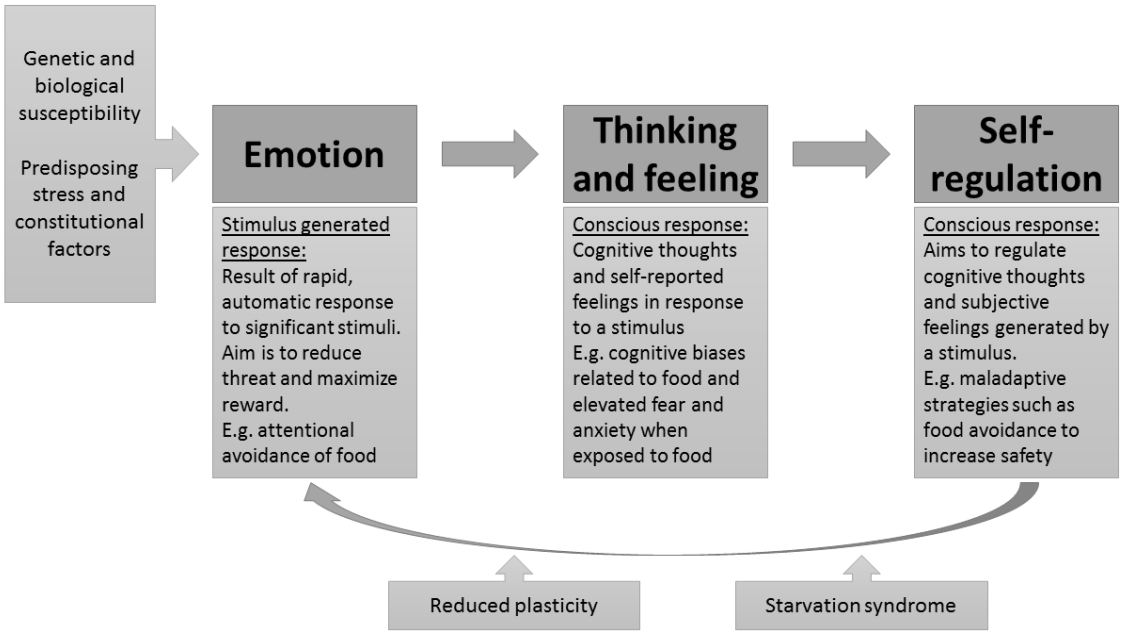


1.5.2 Integrative neuroscience model of anorexia nervosa (Hatch et al., 2010)

The integrative neuroscience model postulates that AN is a disorder of emotion characterised by anomalies in early reactivity to emotional cues, social-cognition and cognitive processing, and in self-regulation (Hatch et al., 2010) (**Figure 4**). According to the model, prior to onset of full illness people with AN show elevated sensitivity to threat and negative emotional cues. Heightened threat sensitivity contributes to the development and maintenance of the illness by exacerbating difficulties in social-

cognition and cognitive processing. These processes, in turn, are believed to fuel disordered eating, which is seen as a self-regulation strategy to reduce negative affect and minimise threat. Over time this is believed to lead to the starvation syndrome, ingrained habits, and reduced neural plasticity. Support for this model comes from functional magnetic resonance imaging (fMRI) studies that have found elevated reactivity in subcortical regions associated with emotional processing, such as the amygdala and insula, as well as anomalies regions associated with cognitive processing and control, including lateral prefrontal cortex (Donofry et al., 2016).

Figure 4. Simplified version of the Integrative neuroscience model of AN.

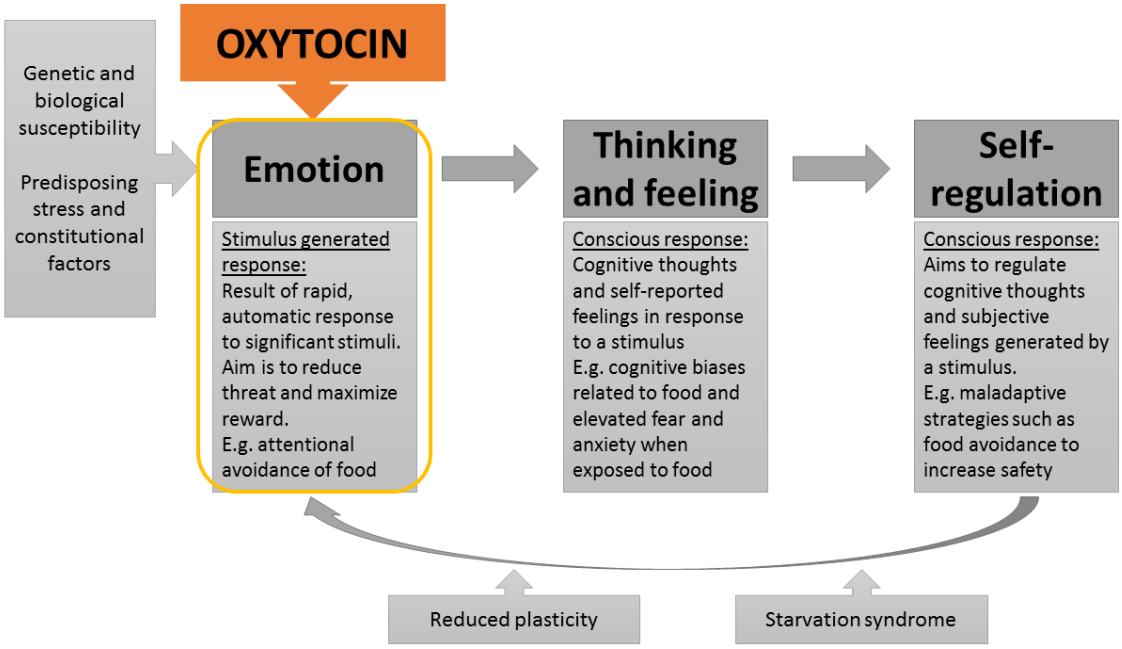


Preclinical studies have demonstrated that oxytocin can not only reduce physiological stress, but also modulate fear- and anxiety-related behaviours in rodents (Yoshida et al., 2009; Parr et al., 2013; Neumann and Slattery, 2016). Emerging research among humans has also suggested that intranasal oxytocin may be effective in targeting threat processing and reducing fear- and anxiety-related

responses (Bertsch et al., 2013; Domes et al., 2013b; Domes et al., 2013a; Kim et al., 2014a). A recent pilot study reported the oxytocin significantly reduced attentional bias towards food related images among people with AN (Kim et al., 2014a). Thus, in the integrative neuroscience model of AN, oxytocin may be beneficial in reducing heightened threat sensitivity and subjective anxiety, ultimately reducing reliance on food avoidance and other core eating disorder behaviours (**Figure 5**).

Chapter 5 in this thesis explores the effects of a single dose of intranasal oxytocin on attentional responses to food images and subjective anxiety in AN.

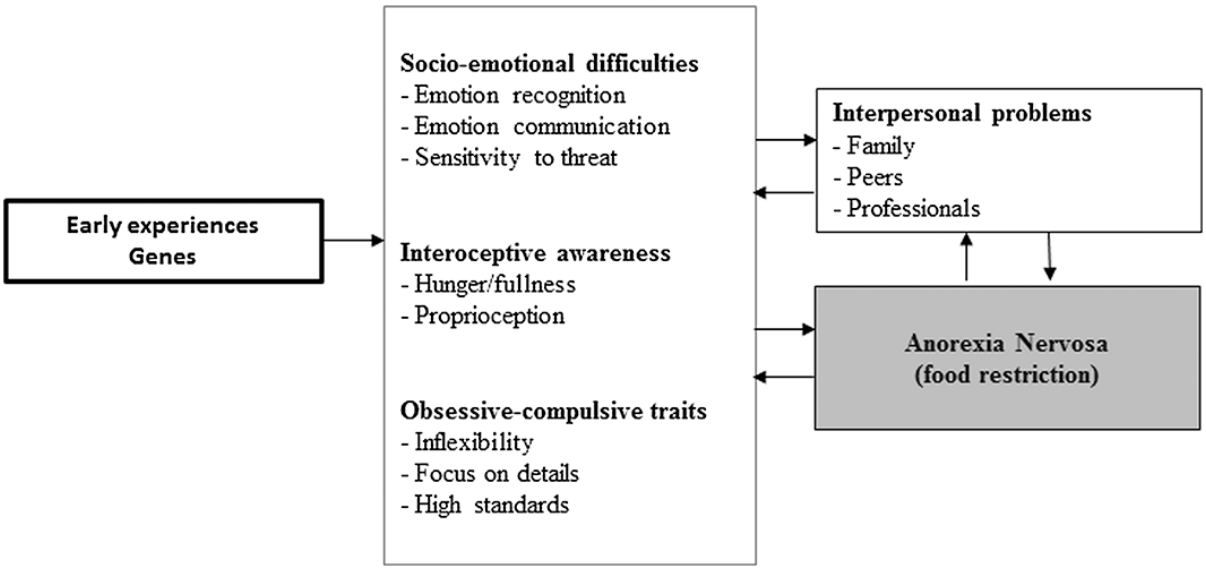
Figure 5. Potential site of oxytocin action in the integrative neuroscience model of AN



1.5.3 Theoretical model of the aetiology of AN (Bruch, 1982; Treasure and Cardi, 2017)

Bruch (1982) highlighted the role of early attachment and social experiences in the development of AN. She proposed that difficult early experiences feed into further anomalies in social-emotional processing and interoception, and ultimately to disordered eating, which in turn perpetuates these anomalies (Bruch, 1982). The original model has recently been updated to include findings from experimental work conducted since the conception of the model and is presented in **Figure 6** (Treasure and Cardi, 2017). The authors found extensive support for the original model and provided a few updates emphasising recent advances in the field (Treasure and Cardi, 2017). For instance, recent work suggests that difficult early experiences and abuse in early life are leading risk factors for a number of psychiatric disorders (Teicher and Samson, 2016; Teicher et al., 2016). These negative experiences are suggested to alter subsequent brain development, increasing anxiety and introducing anomalies in emotional and reward processing (Teicher et al., 2016). Additionally, studies using ecological momentary assessments that have documented that episodes of food restriction in AN are often preceded by interpersonal stress (Engel et al., 2013). These findings suggest that the eating disorder behaviours are used as maladaptive coping mechanisms to deal with elevated anxiety and interpersonal difficulties. Thus, the model highlights that in addition to weight restoration, treatments would benefit from targeting elevated anxiety and threat sensitivity, and difficulties in social-emotional functioning.

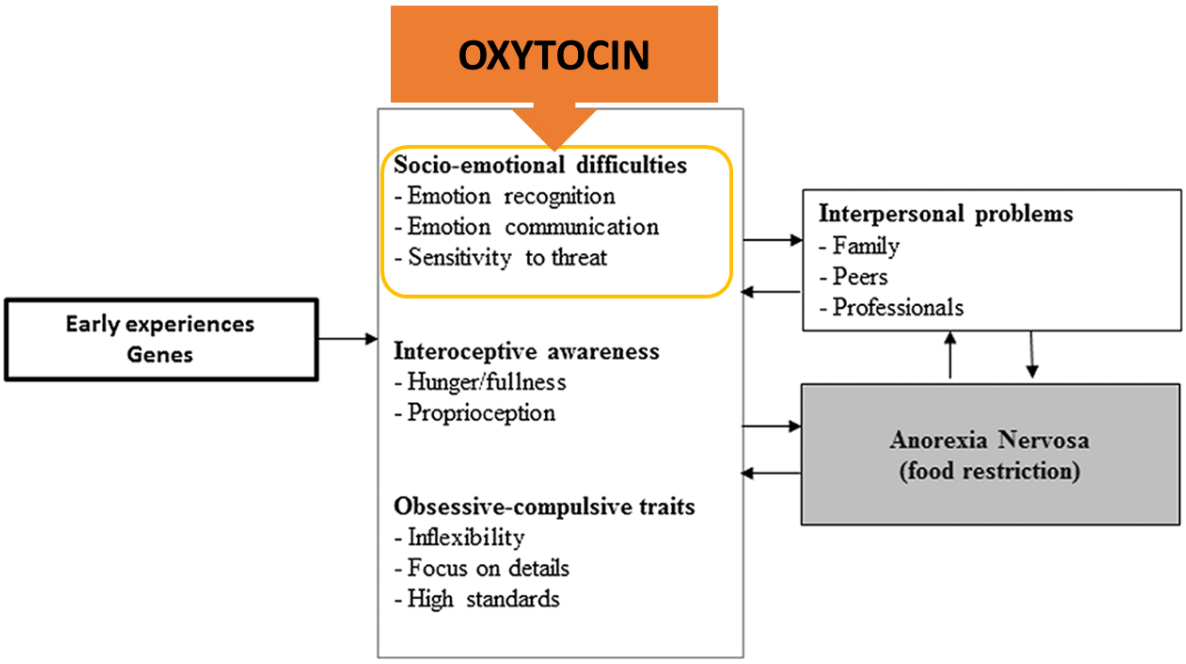
Figure 6. Updated theoretical model of AN based on Bruch (1982) (Treasure and Cardi, 2017)



Within the updated theoretical model of the aetiology of AN, the neuropeptide oxytocin could be beneficial in improving social-emotional functioning (**Figure 7**). Previous work has documented improvements in social cognition, including interpretation of emotions and social communication, following oxytocin administration in healthy individuals as well as among clinical populations (Van Ijzendoorn and Bakermans-Kranenburg, 2012; Bakermans-Kranenburg and van Ijzendoorn, 2013; Shahrestani et al., 2013). By modulating social-emotional difficulties, oxytocin may be beneficial influencing core eating disorder behaviours, which are believed to be used as coping mechanisms to deal with these difficulties.

Chapter 6 in this thesis explores the effects of a single dose of intranasal oxytocin on social-emotional processing in AN.

Figure 7. Potential site of oxytocin action in the theoretical model of the aetiology of AN



1.6 Aims and hypotheses

The overall aim of this thesis was to examine the processes that influence eating behaviour and underlie difficulties in social-emotional processing in AN. Additionally, we aimed to explore the potential of a single dose of intranasal oxytocin as a new intervention targeting the negative valence system in AN. More specifically, we explored whether oxytocin can be used to lower the high cortisol levels, subjective anxiety, and food-related threat sensitivity in AN. We also examined the potential of a single dose of intranasal oxytocin in targeting anomalies in the systems of social processes in AN. Specifically, we examined if intranasal oxytocin could be used to improve difficulties in recognition and expression of emotions in AN. The aims of each chapter are detailed in Table 3.

Table 3. Aims and hypotheses of the studies in this thesis

Chapter	Title	Aims & Hypotheses
Chapter 2	The effects of negative and positive mood induction on eating behaviour: A meta-analysis of laboratory studies in the healthy population and eating and weight disorders	The aim of this meta-analytic review was to examine the effects of experimentally manipulated mood on eating behaviour across eating and weight disorders.
Chapter 3	Blunted neural response to implicit negative facial affect in anorexia nervosa	The aim of this neuroimaging study was to examine the differences between people with AN and healthy comparison participants in the neural substrates employed during implicit processing of positively and negatively valenced adult facial expressions. We hypothesised that participants with AN would have atypical pattern of reduced recruitment of lateral PFC and increased activation in the amygdala, insula, and fusiform gyri while processing emotional faces.
Chapter 4	FMRI Study of Neural Responses to Implicit Infant Emotion in Anorexia Nervosa	The aim of this neuroimaging study was to investigate differences in the neural processes underlying implicit processing of infant emotion in people with AN and healthy comparison participants

Chapter	Title	Aims & Hypotheses
		We hypothesised that while viewing emotional infant faces, people with AN would have atypical increased recruitment in regions involved in emotion down-regulation, namely the bilateral lateral PFC, as well as in regions associated with emotion processing, such as the amygdala and insula.
Chapter 5	The effects of intranasal oxytocin on smoothie intake, cortisol and attentional bias in anorexia nervosa	The aim of this experimental study was to investigate the effects of a single dose of intranasal oxytocin on fruit smoothie intake during a standard laboratory food challenge in people with AN and healthy comparison women. We also investigated the effects of intranasal oxytocin on physiological stress, as measured with salivary cortisol, and attentional bias towards food images before and after the smoothie challenge. We hypothesised that intranasal oxytocin would decrease physiological stress and attentional bias towards food images, and increase smoothie intake in people with AN.
Chapter 6	Effects of Intranasal Oxytocin on the Interpretation and Expression of Emotions in Anorexia Nervosa	The aim of this experimental study was to investigate the effects of a single dose of intranasal oxytocin on interpretation and expression of emotions in people with AN and healthy comparison women.

Chapter	Title	Aims & Hypotheses
		We hypothesised that intranasal oxytocin would improve the accuracy of interpretation of emotions and increase emotion expression in response to emotionally provoking stimuli in people with AN.
Chapter 7	Meta-analytic review of the effects of a single dose of intranasal oxytocin on threat processing in humans	The aim of this meta-analytic review was to pool studies investigating the effects of a single dose of intranasal oxytocin on threat processing, including physiological startle response, attentional bias, and approach and avoidance responses, among healthy and clinical populations. We hypothesised that oxytocin would reduce the sensitivity to threat and impact on the startle response, attentional bias, and avoidance of threatening stimuli.
Chapter 8	Meta-analysis of the effects of intranasal oxytocin on interpretation and expression of emotions	The aim of this meta-analytic review was to pool studies investigating the effects of a single dose of intranasal oxytocin on social-emotional functioning, including emotional theory of mind, recognition of basic emotions, sensitivity to recognise emotions, and emotion expression among healthy and clinical populations. We hypothesised that oxytocin would improve interpretation of basic and complex emotions, and increase emotion expression.

CHAPTER 2:

The effects of negative and positive mood induction on eating behaviour: A meta-analysis of laboratory studies in the healthy population and eating and weight disorders

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Review

The effects of negative and positive mood induction on eating behaviour: A meta-analysis of laboratory studies in the healthy population and eating and weight disorders

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ABSTRACT

Objective: The aim of this study was to conduct a meta-analysis to quantify the effect of induced negative and positive mood on meal consumption in healthy participants and patients with eating and weight disorders.
Method: The search term "MOOD" was combined with the following keywords: "TEST MEAL" or "LABORATORY FEEDING" or "LABORATORY MEAL" or "TASTE TEST" or "TASTE TASK" to identify the relevant studies.
Results: Thirty-three studies were selected, including 2491 participants. Two meta-analyses compared negative mood or positive mood with neutral mood. Induced negative mood was significantly associated with greater food intake, especially in restrained eaters and binge eaters. Positive mood was also associated with greater caloric intake across groups.
Conclusion: These findings support the causal relationship between negative mood and greater food intake, especially in restrained eaters and binge eaters. Preliminary evidence indicates that strategies to improve positive mood might be of benefit for people with anorexia nervosa and bulimia nervosa, although the size of the effect across a single meal is small.

Contents

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0149-7634/

Food intake is not only regulated by metabolic needs (Elmquist et al., 2005; Manning and Batterham, 2014), but is also affected by emotional states, motivations and self-regulatory processes (e.g. Treasure et al., 2011). Negative affect and increased or decreased cognitive control can override the basic maintenance of energy balance (Zheng et al., 2009; Adam and Epel, 2007), with subgroups of individuals reducing or increasing food intake to cope with stress and negative emotions (Epel et al., 2004; Macht, 2008; Stone and Brownell, 1994).

Negative emotions or difficulties with emotional regulation have been found to be transdiagnostic risk and maintenance factors for all forms of eating and weight disorders. Negative affect predicts the future onset of eating disorder symptoms in a large number of prospective studies (Jacobi et al., 2011; Vannucci et al., 2015; Michopoulos et al., 2015); also it mediates the relationship between childhood violence (abuse and bullying) and obesity (Midei and Matthews, 2011). In contrast, positive affect has been found to protect against the development of metabolic syndrome (Midei and Matthews, 2011).

Negative mood as a possible maintaining factor for eating disorders has been examined using daily assessments of negative emotions and eating disorder symptoms in naturalistic settings (ecological momentary assessment – EMA). Studies employing EMA techniques show a consistent association between negative mood and bulimic symptoms in clinical populations (Haedt-Matt and Keel, 2011), with the highest rates of bingeing and purging occurring on days characterised by high negative affect (Crosby et al., 2009). Similar patterns are observed in anorexia nervosa, with an association between restriction and daily labile mood (Engel et al., 2005) and anxiety (Lavender et al., 2013). Studies in populations with tendencies to overeat (i.e. obese individuals) and/or restrict their caloric intake (i.e. restrained eaters) show a less clear association between abnormal eating behaviours and negative mood and report inconsistent findings (e.g. Goldschmidt et al., 2013; Haynes et al., 2003; Yeomans and Coughlan, 2009).

A limitation of the EMA studies is that they can only indicate an association between two variables. In order to test the hypothesis that mood plays a casual role in the regulation of eating behaviours is necessary to use an experimental design in which mood is experimentally shifted and the resultant eating behaviour is observed. The aim of this systematic review and meta-analysis is to quantify the impact of induced negative and positive mood on eating behaviour in laboratory, controlled studies in participants with a range of eating behaviours (healthy controls: HCs, restrained eaters: RE, patients with anorexia nervosa: AN, bulimia nervosa: BN, binge eating disorder: BED, other specified feeding or eating disorder-binge eating subtype: sub-BED, and obese individuals).

1. Methods

1.1. Literature search

The electronic databases AGRIS (1991 – present), Embase (1974 – present), HMIC (1979 – present), International Pharmaceutical Abstracts (1970 – present), Maternity and Infant Care (1971 – present), MEDLINE (1946 – present) and PsycINFO (1806 – present) using OVID; Science Citation Index Expanded (1900 – present), Social Sciences Citation Index (1956 – present) and Arts & Humanities Citation Index (1975 – present) using Web of Knowledge®; and United States National Library of Medicine using PubMed were searched to identify relevant research articles written in English and published in peer reviewed journals during the available years up to February 2015, following the PRISMA guidelines (Moher, 2009).

1.2. Search terms

The search terms "MOOD" was combined with the following keywords: "TEST MEAL" or "LABORATORY FEEDING" or "LABORATORY MEAL" or "TASTE TEST" or "TASTE TASK" or "FOOD INTAKE". We selected the key word "mood" as it typically describes a pervasive and predominant emotional state, compared to "affect" which describes a more sudden and short lived reaction (Ketai, 1975).

1.3. Eligibility criteria

To be included in the meta-analysis studies were required to: (1) investigate the impact of experimentally induced mood on eating behaviour, (2) use procedures to induce a psychological change in mood rather than physiological stress, (3) have a laboratory test-meal during which participants are invited to eat as much as they want to and feel comfortable with, (4) have at least one mood condition and a neutral condition to allow for comparisons (i.e. negative vs. neutral; positive vs. neutral), (5) include an adult population ($\geq$18 years old) of: (a) HCs (i.e. individuals who never suffered from mental illnesses), (b) REs (i.e. individuals who consciously monitor their food intake to remain slim or promote weight loss), (c) participants with a diagnosis of EDs (AN, BN, BED, sub-BED) or (d) obese participants (i.e. Body Mass Index – BMI – $\geq$30), (6) provide sample size and empirical data (mean, standard deviation – SD) on mood ratings before and after mood induction and on the amount of food consumed during the test-meal in order to calculate effect sizes (ESs; principal summary measures) and (7) had a minimum of 15 participants per group.

1.4. Study selection

The initial literature search yielded 7777 papers with 2 additional papers found through personal correspondence. After removing duplicates, 5167 papers were screened based on the abstract and 5097 papers were excluded for not meeting the inclusion criteria. Further 36 papers were excluded for not reporting relevant data or for including heterogeneous groups of REs. Additionally, one paper was excluded for using a supervised test meal. During the test meal, participants admitted to hospital were asked to consume their lunch for the day, rather than subjectively choose the amount and type of foods they wanted to eat after mood induction. Thirty-three studies were finally included in the meta-analysis (see Fig. 1 for a summary of the studies selection process).

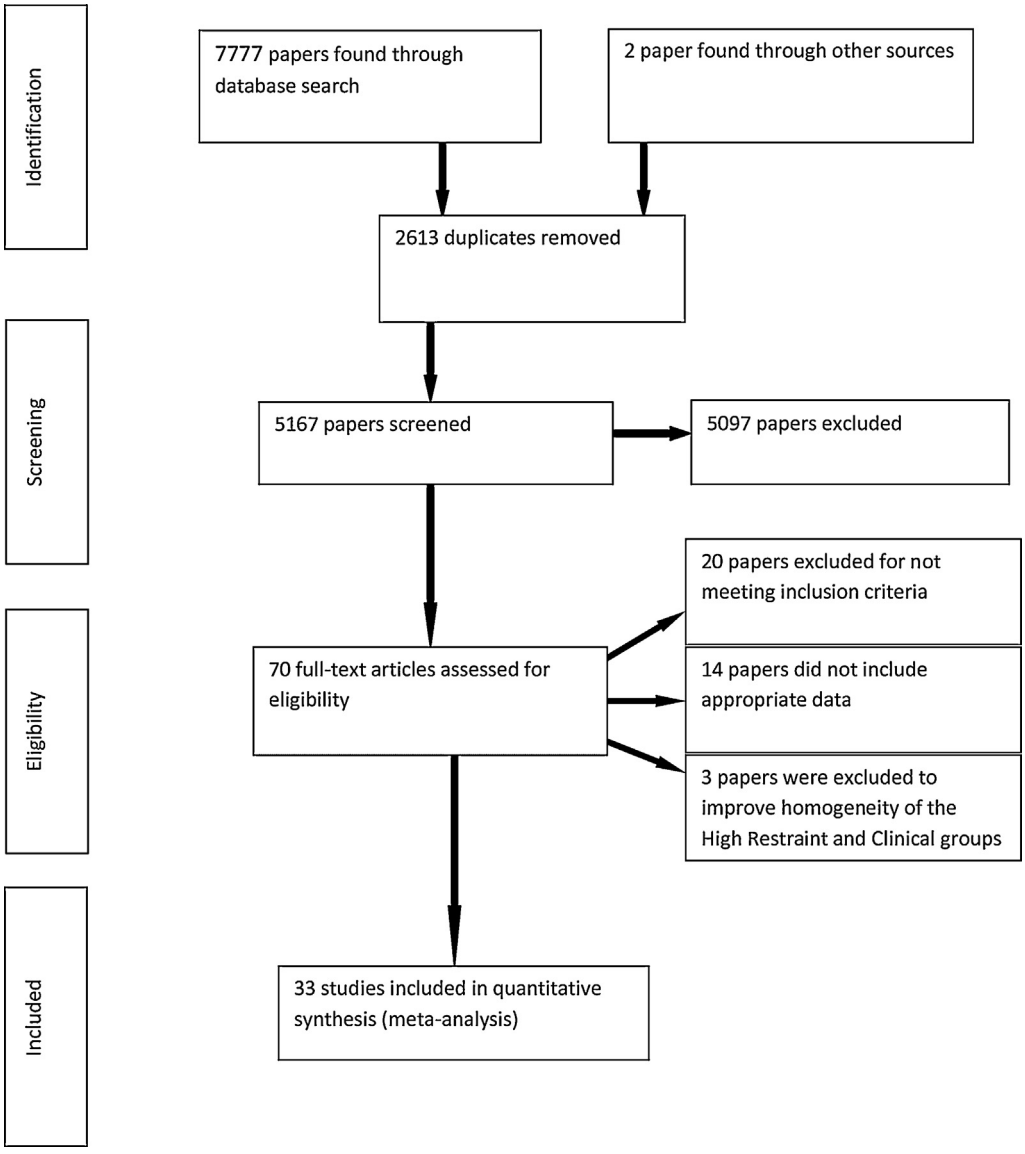

Fig. 1. Study selection. Flow chart of study selection.

J.L. and V.C. carried out the literature searches, final screening and eligibility and criteria compliance assessment. Any cases where eligibility remained uncertain were referred to the whole team for further discussion. When quantitative data were unavailable, the corresponding authors were contacted to request raw data. The following authors provided relevant data through personal correspondence: Appelhans et al. (2011), Bongers et al. (2013), Cardi et al. (2013), Cardi et al. (2015), Cardi et al. (unpublished) and Mittal et al. (2011). References from all relevant articles were inspected for additional studies not yielded by the initial search. One study was found using this method.

1.5. Data collection and synthesis

Information regarding: (1) valence of induced mood (i.e. positive, negative, neutral), (2) mood induction procedure used, (3) participants mood ratings before and after mood induction, (4) sample size, (5) means and SDs for the amount of food consumed during the test meal and (6) types of food on offer (i.e. sweet, savoury) was extracted from the included papers. The following method was undertaken to synthesise the data: where studies included two separate test meals only the test meal that took place during or immediately after mood induction was included. Studies were divided into the following groups considering participants included in the studies: HC, Obese, REs, Clinical (AN, BN, BED, sub-BED). Two meta-analyses were conducted comparing: (1) overall effect of negative vs. neutral mood on food consumption and (2) overall effect of positive vs. neutral mood on food consumption. Meta-regressions were calculated to investigate any heterogeneity arising from the meta-analysis.

1.6. Mood induction procedures

The mood induction methods employed by the studies in the meta-analysis were grouped as follows:

- Others' emotional display (OED) that included: excerpts from movies, TV shows, on purpose made film clips and vignettes, classical music accompanied by a sad story.
- Guided mood induction (GMI) that consisted of interviewing participants or asking them to write about an emotional experience that they could recall.

- Social feedback (SF) that included: providing participants with false, ego-threatening feedback on their performance, facing the threat of public speaking or being subjected to the Trier Social Stress Test (Kirschbaum et al., 1993). In the Trier Social Stress Test three judges trained to maintain a neutral expression request to subjects to prepare and deliver a brief presentation and to complete a mental arithmetic task (Kirschbaum et al., 1993).

1.7. Types of food on offer

Studies were categorised in terms of whether they included offering only sweet, only savoury, both sweet and savoury or a choice of sweet or savoury foods (i.e. participants were asked to choose one of the two options before mood induction) during the test meal (see Table 1). The sweet foods offered included: cookies, chocolate, ice cream, sweet popcorn, chocolate pudding, and fruit smoothies. The savoury foods offered included: popcorn, sandwiches, cheese cubes, crisps, and crackers. The sweet and savoury foods offered included: chocolate, crisps, cookies, pretzels, crackers, peanuts, fresh fruit and vegetables, sandwiches, entire meals (e.g. pasta), and a variety of desserts (e.g. cakes). The choice of sweet or savoury foods offered included: salted popcorn or raisins, crisps or chocolate.

1.8. Statistical analyses

The analyses were performed using Stata 11.0 (Stata Corporation, College Station, TX, USA) with the user-contributed commands *metan* (Bradburn et al., 1998), *metabias*, *metatrim* (Steichen, 1998) and *metareg* (Sharp, 1998). Cohen's *d ES* (i.e. difference between two means divided by the pooled standard deviations) and 95% confidence intervals were reported for all studies. Effect sizes can be interpreted as: negligible (≥ -0.15 and >0.15), small (≥ 0.15 and >0.40), medium (≥ 0.40 and >0.75), large (≥ 0.75 and >1.10), very large (≥ 1.10 and >1.45) and huge (≥ 1.45). When the amount of test meal consumed after the negative or positive mood induction was compared to the amount consumed in the neutral condition, a positive *ES* suggested that participants consumed more food in the experimental condition (negative or positive mood induction). A p value of ≤ 0.05 was considered to be significant.

The meta-analysis was conducted using the random-effects model. The random-effects model assumes that both the within-group variability of scores and mean *ES* are caused by differences between the studies. The homogeneity of the true *ES* was assessed using Cochran's Q statistic and I^2 index (0–1) giving a measure of the magnitude of heterogeneity in the sample of studies (Higgins et al., 2003). Meta-regressions were performed to adjust for variation caused by differences in: (1) methods used to induce negative and positive mood (OED, GMI, SF), (2) type of negative or positive mood induced (positive mood or happiness; negative mood, anger, sadness, anxiety, stress or fear); (3) participant group (HCs, Obese, REs, Clinical); (4) types of food offered (sweet, savoury, sweet and savoury, sweet or savoury). Publication bias was assessed using Begg's adjusted rank test (Begg and Mazumdar, 1994) Egger's test (Egger et al., 1997) and by observing the funnel plots.

2. Results

2.1. Study characteristics

Table 1 presents a summary of the main characteristics of the studies included in the meta-analysis. The Cohen's *d* in Table 1 indicates the standardised difference in outcome measures in the different mood conditions.

2.2. Study participants

The total number of participants was 2491, of which 1772 were HCs, 270 REs, 317 had a diagnosis of EDs (i.e. 33 AN, 23 BN, 156 BED and 105 Sub-BED) and 132 suffered from obesity. The overall mean age of the sample was 24.4 years ($SD = 5.09$) and the overall mean BMI was 24.7 kg/m^2 ($SD = 3.03$).

2.3. Mood manipulation outcome

Sixteen studies reported participants' positive and/or negative mood ratings before and after mood induction (see Supplementary figs. 1 and 2). A further 6 studies provided the relevant data through personal correspondence (see Fig. 1). Eleven studies measured change in positive mood ratings after negative, positive and neutral mood induction (see supplementary figure 1). All studies included a HC group and two studies also included clinical groups (AN, BN). Findings indicated that positive mood was significantly reduced after negative mood induction ($ES = -0.80$, 95% CI -1.08, -0.52, $p < 0.001$) and significantly increased after positive mood induction ($ES = 0.52$, 95% CI 0.26, 0.79, $p < 0.001$). As expected no significant changes were observed in the neutral condition ($ES = 0.082$, 95% CI -0.095, 0.260, *NS*).

Supplementary figure 1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neubiorev.2015.08.011.

Ten studies measured change in negative mood ratings after negative, positive and neutral mood induction (see supplementary figure 2). All studies included a HC group and one study also included a clinical group (AN). The overall findings indicated that negative mood increased significantly after negative mood induction ($ES = 0.89$, 95% CI 0.47, 1.31, $p < 0.001$). Positive mood induction had only a modest effect in reducing negative mood ratings ($ES = -0.28$, 95% CI -0.58, 0.03, $p = 0.073$) and the neutral condition had a small, but significant effect in reducing negative mood ($ES = -0.21$, 95% CI -0.32, -0.10, $p < 0.001$).

Supplementary figure 2 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neubiorev.2015.08.011.

2.4. Food intake: negative vs. neutral mood induction

The overall findings indicated that there was a significant effect of negative mood on food consumption ($ES = 0.260$, 95% CI 0.076, 0.444; $p = 0.006$) with participants consuming more food under this condition. The heterogeneity of the sample was high ($I^2 = 89.1\%$). A meta-regression was conducted to further investigate the variance using the following variables: participant group, mood induction method, type of mood induced and types of food offered. The participant group variable significantly explained some of the variance (Adj. $R^2 = 21.75\%$, $t = 3.26$, $p = 0.002$, 95% CI 0.122, 0.517) indicating that the RE, BED and Sub-BED groups consumed significantly more food when negative mood was induced compared with the HC and obese groups. The type of food offered also significantly explained a small percentage of the variance observed (Adj. $R^2 = 7.88\%$, $t = -2.13$, $p = 0.039$, 95% CI -0.438, -0.118) indicating that more food was consumed in the negative mood condition when participants were offered either sweet or savoury foods than when they were offered both or a choice of sweet or savoury. There was no evidence of publication bias (Begg's test $p = 0.487$, Egger's test $p = 0.288$). Results are shown in Fig. 2.

2.5. Food intake: positive vs. neutral mood induction

The overall findings showed that significantly more food was consumed when positive mood was induced than in the control

Table 1
Studies' characteristics including first author and year of publication, participants' group and gender, mean and standard deviation (SD) of age and body mass index, mood induction procedure used (i.e. others' emotional display: OED, guided mood induction: GMI, social feedback: SF), type of food offered and effect size (Cohen's *d*).

Study	Group: *N* and gender	Age: mean (SD)	BMI: mean (SD)	Mood induction procedure	Mood induced	Type of food offered	Cohen's *d* (95% CI)
Healthy controls							
Appelhans et al. (2011)	HCs *N* = 37 (F/M)	31.8 (10.6)	22.8 (1.7)	GMI	Anger	Sweet and Savoury	0.033 (−0.423, 0.489)
Aubie and Jarry (2009) (Expt. 1)	HCs *N* = 62 (All F)	21.43 (4.5)[a]	26.46 (4.98)[a]	OED	Negative Mood	Sweet	0.092 (−0.260, 0.444)
Aubie and Jarry (2009) (Expt. 2)	HCs *N* = 57 (All F)	22.53 (5.90)[a]	25.10 (4.72)[a]	OED	Negative Mood[a]	Sweet	0.153 (−0.214, 0.521)
					Negative mood[a]		−0.014, (−0.381, 0.353)
Bongers et al. (2013)	HCs *N* = 87 (F = 65 M = 22)	21.6 (2.8)	23.55 (3.94)	OED	Sadness	Sweet and Savoury	0.157 (−0.141, 0.455)
					Happiness		0.211 (−0.087, 0.509)
Cardi et al. (2015)	HCs *N* = 36 (All F)	25.9 (5.0)	21.5 (2.0)	OED	Positive Mood	Sweet	−0.040 (−0.502, 0.422)
Cardi et al. (2013)	HCs *N* = 19 (All F)	NR	NR	OED	Positive mood	Sweet	0.069 (−0.567, 0.705)
Cools et al. (1992)	HC *N* = 91 (All F)	28.6 (8.9)	NR	OED	Negative Mood	Savoury	1.155 (0.841, 1.469)
					Positive Mood		0.596 (0.299, 0.893)
Evers et al. (2009) (Expt. 3)	HC *N* = 37 F	22.84	22.99 (2.97)	GMI	Negative Mood	Sweet and Savoury	0.000 (−0.456, 0.456)
Evers et al. (2009) (Expt. 4)	HC *N* = 57	20.8	21.80 (2.46)	SF	Negative Mood	Sweet and Savoury	−0.787 (−1.168, −0.405)
					Positive Mood		0.068 (−0.299, 0.435)
Evers et al. (2010) (Expt. 1)	HC *N* = 37 (All F)	22.92	23.13 (2.89)	GMI	Negative Mood	Sweet and Savoury	0.010 (−0.445, 0.466)
Evers et al. (2013) (Expt. 1)	HC *N* = 70 (All F)	21.9 (3.3)	21.5 (1.95)	OED	Positive Mood	Sweet and Savoury	0.780 (0.436, 1.123)
Evers et al. (2013) (Expt. 2)	HC *N* = 84 (All F)	21.7 (1.02)	NR	GMI	Negative Mood	Sweet and Savoury	0.702 (0.391, 1.014)
					Positive Mood		0.810 (0.511, 1.110)
Fay and Finlayson (2011)	HC *N* = 30 (All F)	NR	NR	GMI	Negative Mood	Sweet	0.582 (0.065, 1.099)
Heatherton et al. (1991)	HCs *N* = 40 (All F)	NR	NR	SF	Anxiety	Sweet	−0.900 (−1.360, −0.439)
Haynes et al. (2003)	HCs *N* = 20 (All F)	22.6 (1.0)	21.65 (0.85)	SF	Stress	Savoury	1.435 (0.736, 2.134)
Lowe and Maycock (1988)	HC *N* = 60 (All F)	NR	NR	OED	Sadness	Sweet	0.214 (−0.145, 0.573)
Mittal et al. (2011)	HCs *N* = 84 (All F)	21.33 (3.18)	21.23 (1.83)	OED	Sadness	Sweet and Savoury	0.817 (0.502, 1.132)
					Happiness		0.649 (0.339, 0.959)

Table 1 (*Continued*)

Study	Group: *N* and gender	Age: mean (SD)	BMI: mean (SD)	Mood induction procedure	Mood induced	Type of food offered	Cohen's *d* (95% CI)
Oliver et al. (2000)	HCs *N* = 68 (F = 41 M = 27)	25.9 (5.6)	21.85 (2.4)	SF	Stress	Sweet and Savoury	0.202 (−0.232, 0.636)
		26.25 (5.75)	22.4 (2.4)				−0.110 (−0.644, 0.424)
Royal and Kurtz (2010)	HC *N* = 56 (All F)	19.30 (1.11)	21.63 (2.65)	SF	Stress	Sweet and Savoury	0.646 (0.266, 1.026)
Schneider et al. (2010)	HCs *N* = 37 (Gender NR)	31.78 (10.59)	22.79 (1.72)	GMI	Anxiety	Sweet and Savoury	−0.070 (−0.526, 0.385)
					Anger		0.033 (−0.423, 0.489)
Schotte et al. (1990)	HC *N* = 29 (All F)	29.6 (9.9)[a]	23.9 (0.9)[a]	OED	Fear	Savoury	2.544 (1.869, 3.218)
Sheppard-Sawyer et al. (2000)	HC *N* = 19 (All F)	19.2 (3.1)	NR	OED	Sadness	Savoury	−0.518 (−1.165, 0.129)
Sproesser et al. (2014)	HC *N* = 251 (F = 178 M = 73)	24 (6)	23 (3)	SF	Negative Mood	Sweet	−0.038 (−0.213, 0.137)
					Positive Mood		0.082 (−0.151, 0.316)
van Strien et al. (2012) (Expt. 1)	HC *N* = 124 (All F)	21.8 (3.6)	23.3 (3.7)	OED	Sad	Sweet/Savoury	−0.141 (−0.391, 0.108)
van Strien et al. (2012) (Expt. 2)	HC *N* = 47 (All F)	19 (range: 18–27)	21.27 (2.66)	SF	Stress	Sweet and Savoury	0.163 (−0.242, 0.568)
van Strien et al. (2013)	HC *N* = 46 (All F)	19.68 (1.86)	21.27 (2.66)	SF	Stress	Sweet and Savoury	−0.075 (−0.484, 0.334)
van Strien and Ouwens (2007)	HC *N* = 86 (All F)	21.1 (1.88)	23.0 (3.45)	SF	Stress	Savoury	−0.208 (−0.508, 0.092)
Turner et al. (2010)	HCs *N* = 106 (F = 74 M = 32)	23.46 (6.40)	22.97 (4.50)	OED	Positive Mood	Sweet	−0.332 (−0.603, −0.061)
Wallis and Hetherington (2004)	HC *N* = 21 (All F)	24.35 (11.45)	23.8 (4.65)	SF	Stress	Sweet	0.149 (−0.457, 0.755)
Werthmann et al. (2014)	HC *N* = 85 (All F)	20.65 (2.03)	21.88 (2.48)	GMI	Negative Mood	Sweet and Savoury	−0.424 (−0.728, −0.120)
Wilkinson et al. (2013)	HC *N* = 21 (All F)	21.19 (3.12)	21.41 (2.61)	GMI	Anxiety	Sweet	1.002 (0.358, 1.645)
Yeomans and Coughlan (2009)	HCs *N* = 24 (All F)	21.67 (1.33)	21.77 (0.57)	OED	Negative Mood	Sweet/Savoury	−1.298 (−1.923, −0.673)
					Positive Mood		0.337 (−0.233, 0.907)
Obese							
Appelhans et al. (2011)	Obese *N* = 24 F & M	39.9 (11.4)	34.9 (4.0)	GMI	Anger	Sweet and Savoury	−0.105 (−0.671, 0.461)
Jansen et al. (1998)	Obese *N* = 53 (All F)	NR	36.4 (6.0)	OED	Negative Mood	Sweet and Savoury	0.002 (−0.670, 0.675)

Laessle and Schulz (2009)	Obese *N* = 31 (All F)	34.5 (8.1)	37.1 (6.3)	SF	Stress	Sweet	−0.258 (−0.758, 0.242)
Schneider et al. (2010)	Obese *N* = 24 (Gender NR)	38.96 (11.36)	34.91 (4.02)	GMI	Anxiety Anger	Sweet and Savoury	−0.049 (−0.615, 0.517) −0.105 (−0.671, 0.462)
Restraint eaters							
Heatherton et al. (1991)	RE *N* = 35 (All F)	NR	NR	SF	Anxiety	Sweet	1.184 (0.675, 1.693)
Heatherton et al. (1998)	RE *N* = 37 (All F)	NR	NR	SF	Negative Mood	Sweet	0.783 (0.310, 1.256)
Scattolon and Nicki (1995)	RE *N* = 75 (All F)	NR	NR	GMI	Social worry	Savoury	2.178 (1.773, 2.583)
Schotte et al. (1990)	RE *N* = 31 (All F)	29.6 (9.9)[a]	23.9 (0.9)[a]	OED	Fear	Savoury	2.544 (1.869, 3.218)
Wallis and Hetherington (2004)	RE *N* = 17 (All F)	24.4 (9.6)	24.4 (3.3)	SF	Stress	Sweet	0.835 (0.133, 1.538)
Clinical populations							
Aubie and Jarry (2009) (Expt. 1)	Sub-BED *N* = 26 (All F)	21.43 (4.52)[a]	25.74 (5.42)[a]	OED	Negative Mood	Sweet	1.137 (0.549, 1.724)
Aubie and Jarry (2009) (Expt. 2)	Sub-BED *N* = 57 (All F)	22.53 (5.90)[a]	25.90 (5.42)[a]	OED	Negative Mood[a] Negative Mood[a]	Sweet	0.967 (0.579, 1.356) 0.234 (−0.135, 0.602)
Cardi et al. Unpublished	BN *N* = 22 (All F)	24.41 (5.75)	23.43 (6.90)	OED	Positive Mood	Sweet and Savoury	−0.286 (−0.880, 0.308)
Cardi et al. (2015)	AN *N* = 18 (All F)	31.0 (10.0)	16.7 (2.7)	OED	Positive Mood	Sweet	0.550 (−0.149, 1.248)
	BN *N* = 23 (All F)	24.4 (5.7)	23.4 (6.9)				0.064 (−0.514, 0.642)
Cardi et al. (2013)	AN *N* = 18 (All F)	31.5 (11.4)	17 (2.6)	OED	Reduced negative mood/improved mood	Sweet	0.140 (−0.514, 0.794)
Laessle and Schulz (2009)	BED *N* = 17 (All F)	33.6 (8.0)	36.7 (5.7)	SF	Stress	Sweet	−0.258 (−0.758, 0.242)

HCs = healthy controls, RE = restrained eaters, BED = binge eating disorder, sub-BED = subclinical binge eating disorder, AN = anorexia nervosa, BN = bulimia nervosa, *N* = sample size, F = females, M = males, NR = not reported.

[a] Aubie and Jarry (2009, Expt. 2) used two different procedures, weight-related teasing and competence related teasing, to induce negative mood.

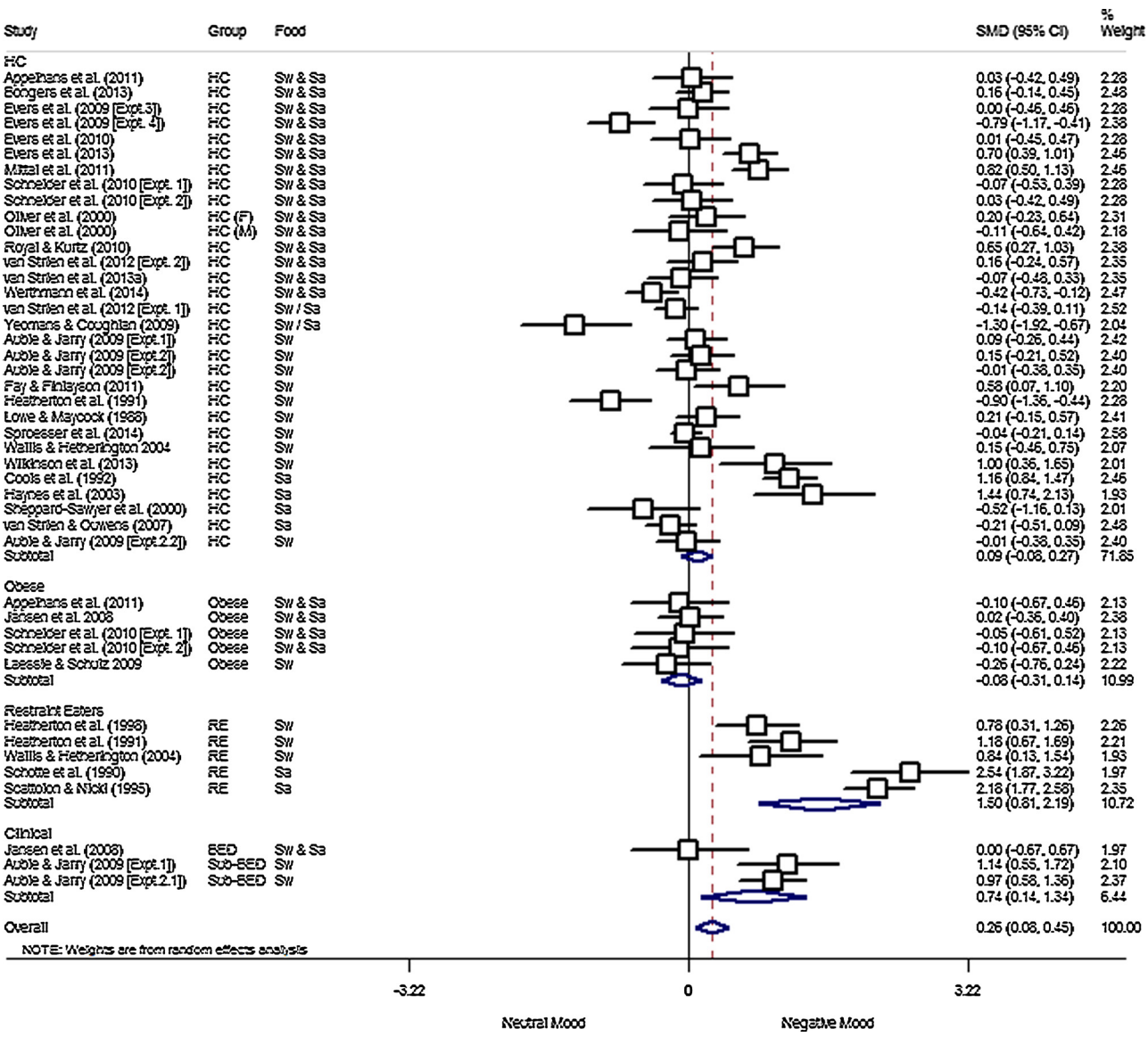

Fig. 2. Amount consumed in the negative vs. neutral mood induction conditions. Forest plot indicating the difference in the amount of food consumed in the negative compared to the neutral experimental conditions. Studies are grouped by participant group. Information regarding the type of mood induced and whether participants were offered sweet and savoury, sweet or savoury, only sweet or only savoury foods is presented on the right. (Sweet and savoury: Sw & Sa, sweet or savoury: Sw/Sa, only sweet: Sw, only savoury: Sa). Effect size is reported as standard mean difference (SMD). EXPT = experiment.

condition (*ES* = 0.261, 95% CI 0.053, 0.469; $p = 0.014$). The heterogeneity of the studies was high ($I^2 = 77.0\%$). A meta-regression was run including the following variables: participant group, mood induction method, type of mood induced and types of food offered. The types of food offered variable approached significance explaining some of the variance observed (Adj. $R^2 = 28.48\%$, $t = 1.91$, $p = 0.079$, 95% CI −0.022, 0.359). This finding indicates that when positive mood was induced there was a trend for more food to be consumed in studies where participants were offered both sweet and savoury food. There was no evidence of publication bias (Begg's test $p = 0.843$, Egger's test $p = 0.838$). Results are shown in Fig. 3.

3. Discussion

The aim of this meta-analysis was to quantify the impact of induced negative and positive mood on eating behaviour in laboratory, controlled studies in participants on the spectrum ranging from normal to at risk and clinical eating behaviour. Findings indicated that negative mood induction was significantly associated with greater food intake across groups, with a more pronounced effect in restrictive eaters and patients with BED or sub-threshold BED. Positive mood was also associated with greater caloric intake across the limited number of groups studied.

The causal relationship between induced negative mood and increased food consumption in binge eaters is in line with the associations found in the literature and confirms the predictions of theoretical models explaining the onset and maintenance of bulimic symptoms in eating disorders (Fairburn et al., 2003; Stice et al., 1998). The mechanism of this effect may be a depletion of self-regulatory abilities caused by emotional distress (Baumeister et al., 1993).

Experimental data on the impact of induced positive mood on eating behaviour in clinical populations were surprisingly limited. Only 3 studies included participants with AN or BN and found that a motivational video-clip (Cardi et al., 2013) and positive mood induction (Cardi et al., 2015; Cardi et al., unpublished) were associated with increased calories consumption in AN and reduced consumption of high palatable, snack foods in BN.

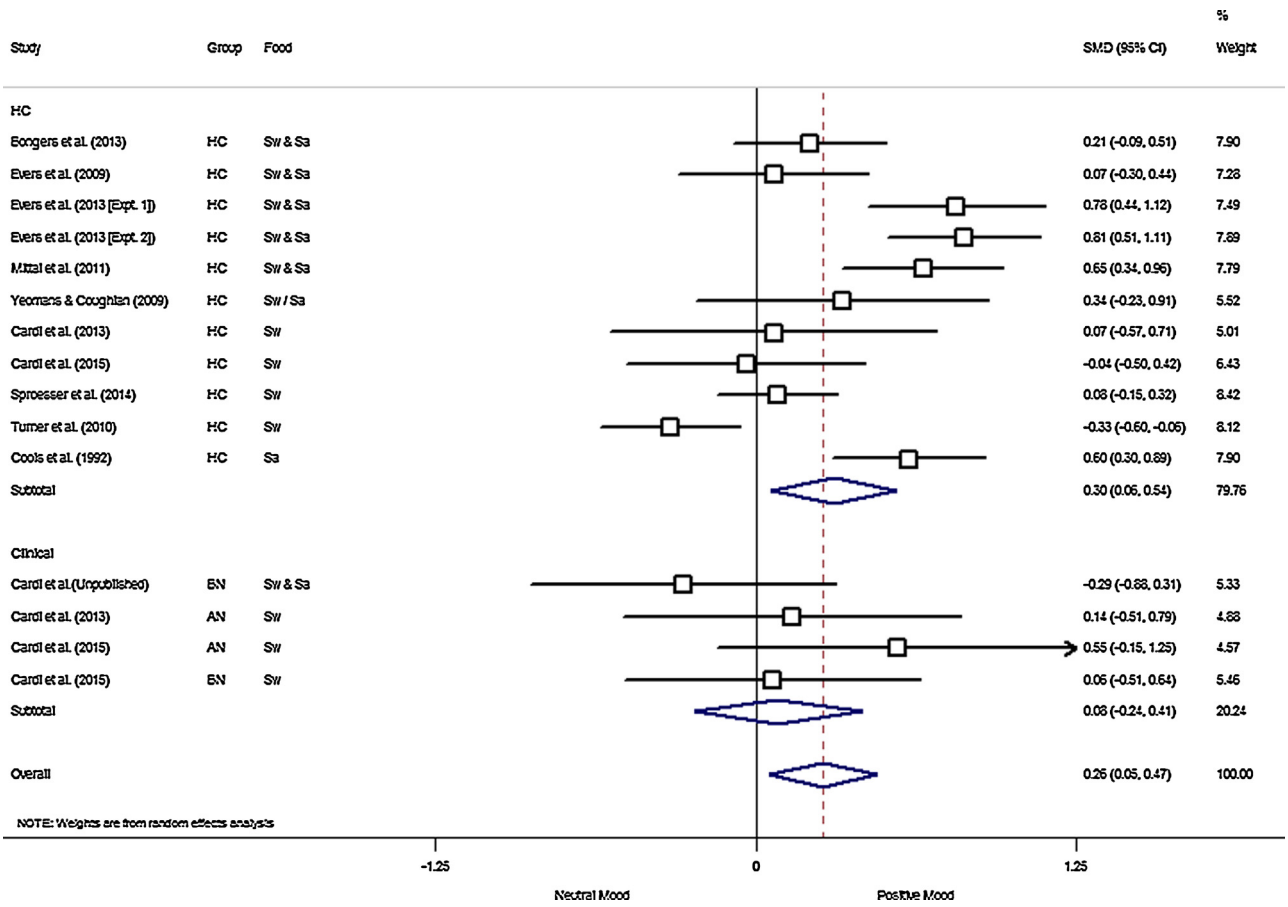

Fig. 3. Amount consumed in the positive vs. neutral mood induction conditions. Forest plot indicating the difference in the amount of food consumed in the positive compared to the neutral experimental conditions. Studies are grouped by participant group. Information regarding the type of mood induced, and whether participants were offered sweet and savoury, sweet or savoury, only sweet or only savoury foods is presented on the right. (Sweet and savoury: Sw & Sa, sweet or savoury: Sw/Sa, only sweet: Sw, only savoury: Sa). Effect size is reported as standard mean difference (SMD). EXPT = experiment.

The finding that positive mood was associated with greater food consumption in HCs corroborates naturalistic studies in which healthy people have shown to engage in hedonic eating during times of joy and happiness (Macht, 1999). Furthermore, experimentally induced positive mood has been shown to increase experienced pleasantness and taste of chocolate in a laboratory setting (Macht et al., 2002).

More food was consumed in the positive mood condition when participants were given both sweet and savoury food to sample, as expected according to the mechanism of sensory specific satiety (i.e. decline in satisfaction derived from consuming one type of food for a long time; Havermans, 2012), Surprisingly, in the negative mood condition more food was consumed when only savoury or sweet foods were offered.

3.1. Limitations

The main limitation of this work is that a wide range of outcome measures (different forms of test meals) and mood induction procedures have been used. This makes the comparison of findings and their interpretation difficult, particularly as it is known that the type of food and setting can have a large effect on food consumption (Wallis and Hetherington, 2009). There have been surprisingly few studies that have used clinical samples. This seems to be a missed opportunity for applying an experimental medicine approach to identify possible novel clinical interventions for eating and weight disorders. The use of standardised outcomes and experimental procedures can improve research in this area.

3.2. Implications and future directions

This meta-analysis corroborates the previous literature suggesting a possible role of negative affect as a transdiagnostic causal risk and maintaining factor for disorders of weight and eating. It follows that strategies to counteract negative mood (e.g. positive mood induction procedures) or to develop effective coping strategies to deal with negative emotions might be useful adjuncts to treatment for these conditions. The preliminary findings from 3 studies that tested positive mood induction in patients with AN or BN are promising, however the effect sizes are small for an individual meal. It would be of interest to know if the effects could be cumulative, if positive mood induction was repeated over time.

3.3. Conclusion

A causal relationship between induced negative mood and abnormal eating behaviour was supported by this meta-analysis. Strategies to counteract negative mood (e.g. positive mood induction) or improve emotion regulation might be of benefit in breaking unhelpful eating habits in people with eating and weight disorders. However, more standardised experimental studies are needed in this area.

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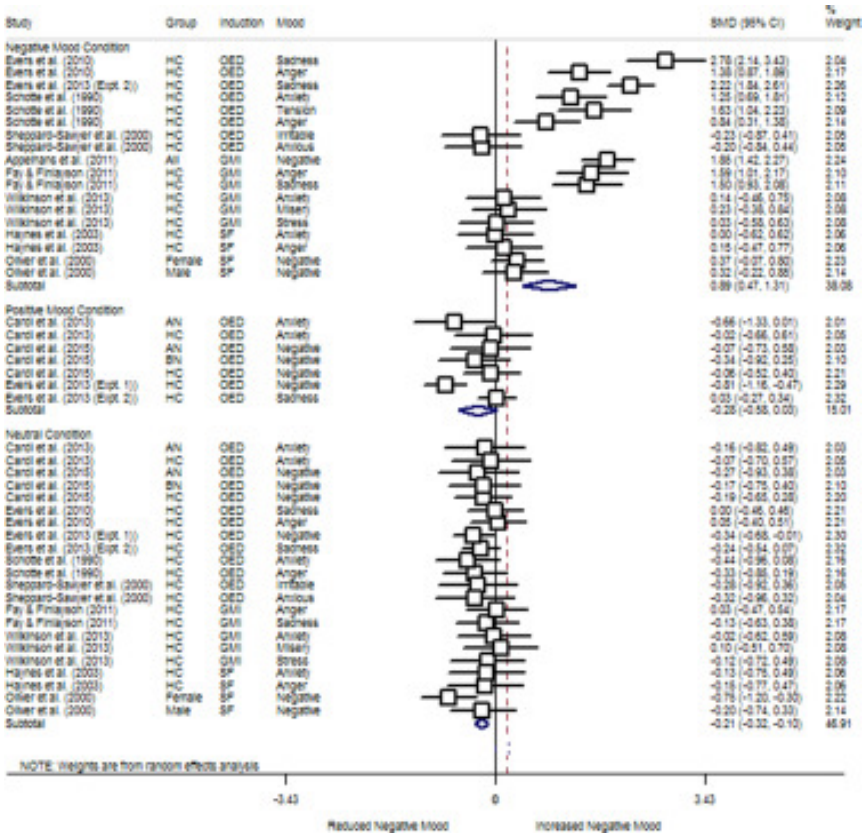
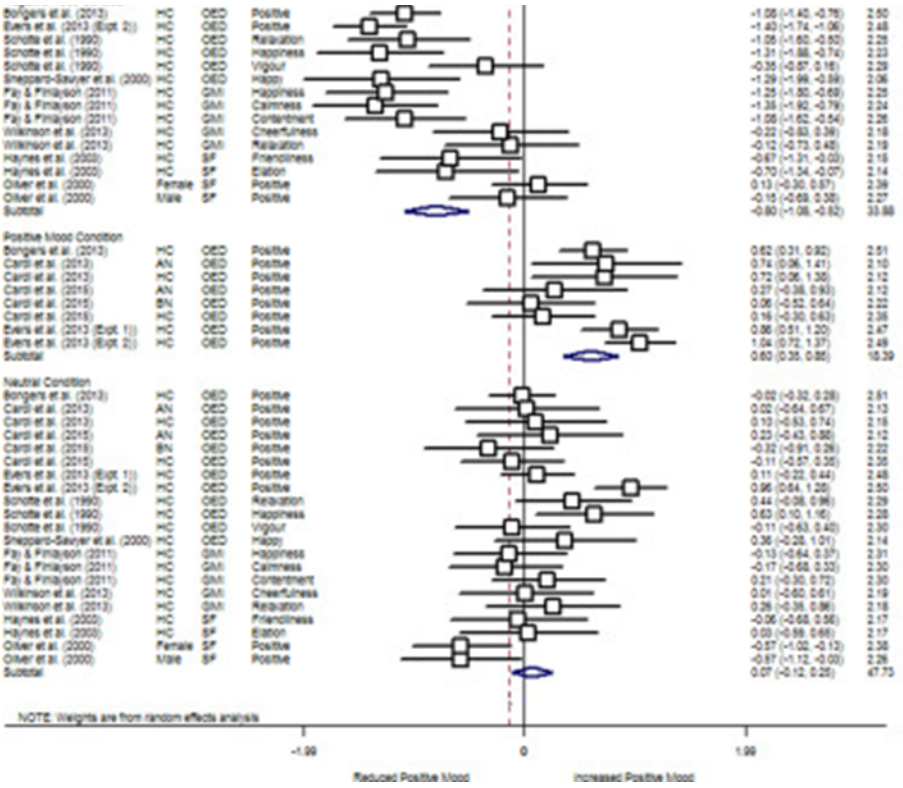
Supplementary Figure 1. Change in positive mood ratings after negative, positive or neutral mood induction



Forest Plot indicating the difference in positive mood ratings after negative, positive or neutral mood induction. Studies are grouped by condition (negative mood condition, positive mood condition, neutral condition). Information regarding participant group, mood induction method, and mood measured before and after induction are presented on the right. Effect size is reported as standard mean difference (SMD). Anorexia nervosa = AN, bulimia nervosa = BN, healthy control = HC, combined scores from all participants = all, others' emotional display = OED, guided mood induction = GMI, social feedback = SF, EXPT = experiment.

Supplementary Figure 2. Change in negative mood ratings after negative, positive or neutral mood induction.



Forest Plot indicating the difference in negative mood ratings after negative, positive or neutral mood induction. Studies are grouped by condition (negative mood condition, positive mood condition, neutral condition). Information regarding participant group, mood induction method, and mood measured before and after induction are presented on the right. Effect size is reported as standard mean difference (SMD). Anorexia nervosa = AN, bulimia nervosa = BN, healthy control = HC, combined scores from all participants = all, others' emotional display = OED, guided mood induction = GMI, social feedback = SF, EXPT = experiment.

CHAPTER 3:

Blunted neural response to implicit negative facial affect in anorexia nervosa

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Blunted neural response to implicit negative facial affect in anorexia nervosa

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ABSTRACT

Background: People with anorexia nervosa (AN) have difficulties in a wide range of social-emotional processes. Previous work suggests atypical involvement of the prefrontal cortex (PFC), amygdala, insula, and fusiform gyri during social-emotional processing in AN.
Methods: Twenty women with AN and twenty healthy comparison (HC) women were presented with happy, fearful, and neutral faces during a functional magnetic resonance imaging study. Group differences were investigated in the following regions of interest: lateral PFC, amygdala, insula, and fusiform gyri.
Results: The HC participants showed significantly increased recruitment of the ventrolateral PFC and amygdala in the fearful > neutral contrast relative to the AN participants. The AN participants showed a significantly increased recruitment of a small cluster in the right posterior insula in the happy > neutral contrast.
Conclusions: These findings are in line with the hypothesis that people with AN have a blunted response to negative and atypical exaggerated response to positive emotionally provoking stimuli.

1. Introduction

Difficulties in social-emotional processing are believed to play an important role both in the onset and maintenance of anorexia nervosa (AN) (Treasure, Corfield, & Cardi, 2012; Treasure & Schmidt, 2013). A wide range of anomalies in social-emotional processing and reactivity to emotional stimuli have been documented in people with acute AN (Bora & Köse, 2016; Caglar-Nazali et al., 2014; Davies et al., 2016). Furthermore, longitudinal cohort studies have reported that difficulties in social cognition and social communication at admission are important predictors of poor treatment outcome at 3- to 18-year follow-up (Nielsen et al., 2015; Speranza, Loas, Wallier, & Corcos, 2007). Thus, these difficulties may contribute to the maintenance of the illness and further understanding of these processes is of importance.

Behavioural studies have documented difficulties in a range of different aspects of social-emotional processing in acute AN (Bora & Köse, 2016; Caglar-Nazali et al., 2014; Davies et al., 2016). Some experimental studies have suggested difficulties in explicit processing of social-emotional cues, such as recognition of facial expressions, in AN (Caglar-Nazali et al., 2014; Oldershaw et al., 2011). However, more recent studies have suggested that these difficulties may be driven by anomalies in interpretation and reactivity to social-emotional cues in AN (Ambwani et al., 2015; Dapelo, Surguladze, Morris, & Tchanturia, 2016). Indeed, people with AN perceive emotionally provoking stimuli to be more negative and colder than healthy comparison (HC) participants (Ambwani et al., 2015; Cardi et al., 2014). Additionally, relative to HCs, people with AN display reduced facial affect in response to emotionally provoking stimuli (Davies et al., 2016). Taken together, these findings suggest that there may be specific anomalies in implicit processing and reactivity to social-emotional cues in AN and further exploration of the neural mechanisms that underlie these processes may be of interest.

Few studies have examined the neural processes that underlie difficulties in social-emotional processing and reactivity to emotional stimuli in people with AN. A recent review reported reduced response in prefrontal regions, including the lateral and medial prefrontal cortex (PFC), while viewing social behaviour in acute AN (McAdams & Smith, 2015). Further, one of the included studies found that reduced response in the lateral and medial PFC to social behaviour at admission to treatment was associated with poorer outcome at discharge (Schulte-Rüther, Mainz, Fink, Herpertz-Dahlmann, & Konrad, 2012). A recent study investigating implicit processing of happy faces of increasing intensity found greater linear increase in activation of the fusiform gyrus in people with acute AN relative to HCs (Fonville, Giampietro,

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Surguladze, Williams, & Tchanturia, 2014). Another study, using a more explicit task, found reduced amygdala response in people recovered from AN relative to HCs in response to negative facial expressions when the faces were coupled with the congruent emotion label (Bang, Ro, & Endestad, 2016). Despite the relative paucity of research in this field, these findings suggest possible anomalies in the recruitment of frontal, amygdala, and visual attentional regions in social-emotional processing in AN.

More work has been conducted in anxiety and mood disorders, which are common comorbid disorders in AN and share similar difficulties in social-emotional processing and reactivity to emotional stimuli (Davies et al., 2016; Hambrook, Brown, & Tchanturia, 2012; Treasure, Stein, & Maguire, 2015). A recent meta-analysis found that relative to HCs, people with depression showed hyperactivation of regions associated with appraisal including amygdala, insula, and fusiform gyrus, during implicit processing of negative facial affect (Groenewold, Opmeer, de Jonge, Aleman, & Costafreda, 2013). Additionally, increased activation of these regions was associated with reduced recruitment of regions associated with emotion down-regulation, including the lateral PFC (Groenewold et al., 2013). Similarly, people with generalised and social anxiety disorders show reduced activation of lateral PFC and associated hyperactivation of the amygdala when processing negative emotional stimuli including negative facial expressions (Mochcovitch, da Rocha Freire, Garcia, & Nardi, 2014). Taken together, these findings suggest that there may be a deficit in prefrontal down-regulation and limbic up-regulation of negative emotion associated with depression and anxiety.

The aim of the current study was to examine the neural substrates employed during implicit processing of positively and negatively valenced facial expressions in AN and HC participants. Based on the neuroimaging findings outlined above, we hypothesised that participants with AN would have atypical pattern of reduced recruitment of lateral PFC while processing emotional faces. Further, based on previous work conducted in mood and anxiety disorders as well as behavioural work in AN, we hypothesise that participants with AN would show a pattern of activation suggestive of anomalies in emotional reactivity. Specifically, we hypothesise that relative to the HCs, participants with AN would show increased activation in the amygdala, insula, and fusiform gyri while processing the emotional facial expressions.

2. Materials and methods

2.1. Participants

Forty right-handed females participated in the study. Twenty participants had a current DSM-IV diagnosis of AN, which was confirmed using the Structured Clinical Interview for Diagnosis – Researcher Version (Spitzer, Williams, Gibbon, & First, 1992). Fifteen participants with AN were recruited from the community through advertisements posted on eating disorder charity websites (i.e. BEAT and Succeed). The AN participants recruited from the community were not receiving psychological treatment during the time of the study. Five participants with AN were recruited from the Bethlem Royal Hospital, South London and Maudsley NHS Trust and were receiving treatment during the study. 60% of the participants with AN were taking antidepressants during the time of the study, 45% of the AN participants reported comorbid depression and 25% reported comorbid anxiety disorder. Twenty age-matched HC women of healthy weight were recruited from the community and amongst King's College London students and staff.

The exclusion criteria for all participants were a history of head trauma, hearing or visual impairment, neurological disease, MRI incompatibility, acute suicidality, and history of (or current) alcohol or drug abuse. Additionally, HC participants were screened with the Structured Clinical Interview for Diagnosis – Researcher Version (Spitzer et al., 1992) and were excluded if they had current or a history of psychiatric disorders. HC participants were also excluded if did not have BMI between 18.5 and 25.0 or were taking psychotropic medication. All participants gave a written, informed consent prior to taking part in the study and were compensated for their participation. The study was approved by a National Research Ethics Service committee (approval number: 11/LO/0373) and was conducted in accordance with the latest version of Declaration of Helsinki.

2.2. Questionnaire measures

The eating disorders examination questionnaire (EDEQ) (Fairburn & Beglin, 1994), a 36-item self-report measure, was used to assess eating restraint, eating concern, shape concern, and weight concern over the past 28 days.

The depression, anxiety, and stress scale (DASS) (Lovibond & Lovibond, 1995), a 21-item self-report measure, was used to assess depression, anxiety, and stress over the past two weeks.

2.3. Design and procedure

During a 6-min event-related functional magnetic resonance imaging (fMRI) task participants were presented with black and white images of faces (Fig. 1). The face stimuli consisted of prototypical happy (intensity: 100%), prototypical fearful (intensity: 100%), and neutral faces. The stimuli were selected from a standardised set of facial expressions (Ekman & Friesen, 1976), and consisted of ten different adults displaying each of the selected emotions (5 female and 5 male). Happy and fearful faces were used as they have previously been found to strongly capture participants' attention and produce robust activation of the amygdala, fusiform gyrus, insula, and prefrontal regions (Fusar-Poli et al., 2009;

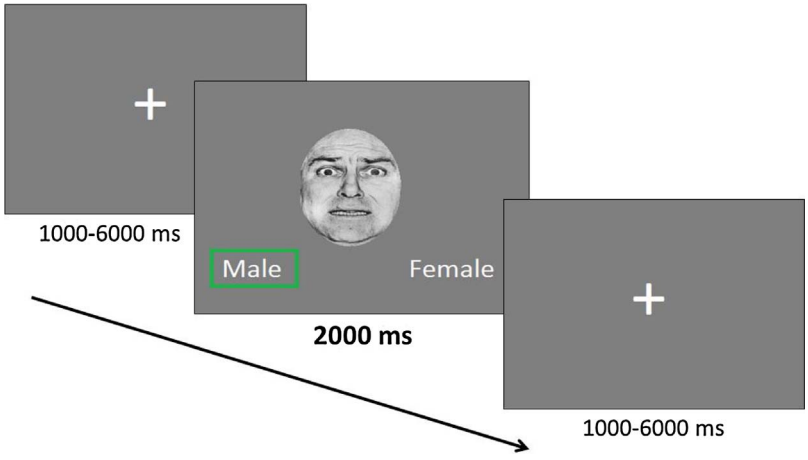

Fig. 1. Implicit emotion processing task.
The happy, fearful, and neutral faces were presented one at a time for 2000 milliseconds (ms) during which participants identified the gender of each face. Faces were preceded by a fixation cross the presentation of which was jittered between 1000 and 6000 ms.

Lundqvist & Ohman, 2005; Williams & Mattingley, 2006). Additionally, unlike angry or disgusted faces, fearful faces are not considered to be indicative of direct threat (Hunnius, de Wit, Vrins, & von Hofsten, 2011). The neutral faces were used as a baseline comparison condition to allow investigation of neural responses to facial affect.

Twenty happy, fearful, and neutral faces were presented in a pseudo-randomised order. The faces were presented one at time for 2000 ms followed by an inter-stimulus interval (ISI) fixation cross. The ISI was varied between 1 and 6 s. To ensure participants were attending to the stimuli, they were asked to identify the gender of the faces by pressing the appropriate buttons. This is a paradigm frequently employed to investigate implicit social-emotional processing (Stuhrmann, Suslow, & Dannlowski, 2011).

2.4. Image acquisition and pre-processing

Magnetic resonance images were acquired using GE Signa 1.5 T scanner (GE Medical Systems, Milwaukee, Wisconsin) at the Centre for Neuroimaging Science, King's College London. An 8-channel headcoil was used to transmit and receive the radio frequency signal. T2* – weighted images depicting blood oxygen level dependent (BOLD) signal were acquired using an interleaved ascending acquisition order with a repetition time of 2 s with 40 ms echo time and an 80° flip angle. Whole brain coverage was acquired in 30 slices with 4 mm slice thickness and 0.4 mm slice gap. 180 T2* – weighted whole brain volumes were acquired with an in-plane resolution of 3.75 mm × 3.75 mm. Additionally, high-resolution sagittal T1–weighted structural images (MP-RAGE) were acquired with a repetition time of 8.592 ms. The echo time was 3.8 ms with an 8° flip angle. Whole brain coverage was acquired in 180 slices with an in-plane resolution of 1.25 mm × 1.25 mm, and 1.2 mm slice thickness and 1.2 mm slice gap.

Prior to analysis, the fMRI data was pre-processed using SPM8 (Wellcome Department of Cognitive Neurology, Institute of Neurology, London) implemented in Matlab 2015b (Mathworks, Natick, Mass.). Pre-processing involved slice-timing and volume-to-volume head motion correction. The data was then co-registered to a high-resolution DARTEL template, created out of the participants T1–weighted structural images (Ashburner, 2007), and normalised to Montreal Neurological Institute (MNI) space. The data was spatially smoothed using an 8 mm FWHM three-dimensional isotropic Gaussian kernel.

2.5. Statistical analysis

2.5.1. Questionnaire and behavioural data

Questionnaire and behavioural data were analysed with Stata 14 (StataCorp. 2015, College Station, TX: StataCorp LP.). Due to skewed nature of the questionnaire data, group differences were investigated with nonparametric median Chi^2 tests. Prior to analysis, gender identification accuracy data was transformed with arcsine and square root transformation to allow further analysis under the general linear model framework. The gender identification accuracy and reaction time data were then analysed with a bootstrapped mixed model (1000 repetitions). Trial (fearful, happy, neutral) and group (AN, HC) were entered as fixed effects variables with a random intercept. Significant effects and interactions were further investigated by calculating post-hoc contrasts and pair-wise comparisons. $P < 0.05$ was considered significant.

2.5.2. Neuroimaging data

On subject level, each participant's fMRI data were analysed under the general linear model framework in SPM 8. A canonical haemodynamic response function was used to model the BOLD signal for each of the following conditions: happy faces, fearful faces, and neutral faces. The time-series was adjusted for head motion using the Friston 24-parameter model (Friston, Williams, Howard, Frackowiak, & Turner, 1996) and low-frequency drift was filtered out using high-pass filter set to 1/128s. To investigate relative activation or deactivation in response to fearful and happy facial expressions compared to neutral faces, the following contrast images were generated for each participant: fear > neutral and happy > neutral.

Each participant's contrast images were then entered into group level analysis, which was conducted with the Robust Regression toolbox (http://wagerlab.colorado.edu/tools) implemented in Matlab 2015b (Wager, Keller, Lacey, & Jonides, 2005). The Robust Regression toolbox uses iteratively re-weighted least squares (IRLS) to increase statistical power and reduce the impact of extreme outliers (Fritsch et al., 2015; Wager et al., 2005). We chose this method to reduce the likelihood of false findings due to presence of extreme outliers that could arise from head motion, scanner related artefacts, or individual participants who were substantially different from the rest of the sample during the time of the MRI scan.

The contrast images were first entered into region of interest (ROI) IRLS analyses to test a priori hypotheses. The WFU Pickatlas toolbox in SPM 8 was used to create the following ROI masks: bilateral amygdala, bilateral insula, bilateral fusiform gyri, and bilateral lateral PFC. To investigate group differences within these regions, group status was added as a contrast-coded covariate (1, −1: AN, HC). Thus, positive test statistic indicates greater activation in the AN group relative to the HC group and negative test statistic indicates greater activation in the HC group relative to the AN group. The ROI findings were corrected for multiple comparisons with a voxel-wise nonparametric permutation test with 10000 iterations ($\alpha < 0.05$). The permutation test uses the max T distribution to generate a new corrected t-threshold each voxel must reach to be considered significant.

We, additionally, explored whether the mean activation in the significant ROIs correlated with medication status, BMI, DASS total score, duration of illness, and eating disorder psychopathology as measured by EDEQ within the AN group. The correlations were conducted by extracting mean signal change from the significant ROIs in each contrast as recommended by Vul, Harris, Winkielman, and Pashler (2009). Nonparametric Spearman's rho correlations were then conducted with Matlab 2015b and $p < 0.01$ was considered significant following correction for multiple comparisons.

Finally, we also conducted an exploratory whole brain analysis to investigate if significant group differences were present in any further regions outside of the a priori ROI masks. As above, the contrast images were entered into whole brain IRLS analysis with group status was added as a contrast-coded covariate (1, −1: AN, HC). The whole brain findings were corrected for multiple comparisons with voxel level False Discovery Rate (FDR) set at $q < 0.05$. Effect size, and lower and upper bound 99.9% confidence interval maps were generated using the EScalc toolbox implemented in Matlab 2015b (http://restfmri.net) (Gao & Zang, 2015).

3. Results

3.1. Clinical characteristics

The socio-demographic and clinical characteristics of the groups are presented in Table 1. There were no significant differences in age or years of education between the AN and HC participants. As expected the participants with AN had significantly lower BMI and reported significantly more eating disorder psychopathology, depression, anxiety, and stress than the HC participants.

3.2. Gender identification

Gender identification mean reaction times and accuracy are presented in Table 2. There was a significant difference between the groups in gender identification RTs, with the HC participants being significantly faster to identify the gender of the faces than the participants with AN.

Table 1
Demographic and clinical characteristics.

	AN (N = 20) Mean (SD)	HC (N = 20) Mean (SD)	X^2 statistic, p value
Age	28.6 (10.81)	25.75 (3.40)	$X^2 < 0$, p = 1.00
Years of education	15.95 (2.56)	16.25 (4.28)	$X^2 = 0.10$, p = 0.752
Duration of illness (years)	13.0 (10.1)	–	–
BMI	15.87 (1.70)	21.15 (2.26)	$X^2 = 27.92$, p < 0.001
EDEQ Total	4.16 (0.99)	0.57 (0.54)	$X^2 = 34.11$, p < 0.001
EDEQ Restraint	4.20 (1.35)	0.45 (0.53)	$X^2 = 26.95$, p < 0.001
EDEQ Eating concern	3.71 (1.06)	0.13 (0.18)	$X^2 = 34.11$, p < 0.001
EDEQ Shape concern	4.66 (1.15)	1.05 (0.98)	$X^2 = 26.95$, p < 0.001
EDEQ Weight concern	4.07 (1.51)	0.65 (0.70)	$X^2 = 20.63$, p < 0.001
DASS Total	70.32 (22.56)	12.30 (13.01)	$X^2 = 27.92$, p < 0.001
DASS Anxiety	17.79 (7.54)	1.80 (3.11)	$X^2 = 16.01$, p < 0.001
DASS Depression	25.47 (11.03)	2.90 (4.38)	$X^2 = 21.55$, p < 0.001
DASS Stress	27.05 (8.34)	7.60 (7.13)	$X^2 = 16.01$, p < 0.001

AN = anorexia nervosa; HC = healthy comparison; BMI = body mass index; EDEQ = Eating Disorder Examination Questionnaire; DASS = Depression, Anxiety, and Stress Scale.

In gender identification accuracy, there was a significant difference between trials with all participants being significantly more accurate in the happy trials than fearful or neutral trials (Z = 2.37, p = 0.018, 95% CI [0.005, 0.05]; Z = 2.73, p = 0.006, 95% CI [0.01, 0.04]). There were no significant differences in accuracy between neutral and fearful trials across participants (Z = 0.33, p = 0.742, 95% CI [-0.02, 0.03]). There was also a significant effect of group and a significant trial x group interaction (Table 2). Post-hoc tests revealed that HC participants were significantly more accurate than the participants with AN only in the fearful trials (Z = 2.64, p = 0.008, 95% CI [0.01, 0.09]), but not in the happy (Z = −1.42, p = 0.155, 95% CI [-0.04, 0.01]) or neutral trials (Z = 1.27, p = 0.205,95% CI [-0.01,0.04]).

3.3. Regions of interest findings

3.3.1. Happy > neutral faces

The ROI findings are presented in Fig. 2 and Table 3. There was a significant difference in BOLD signal change between groups in recruitment of the right posterior insula while viewing happy > neutral faces (Fig. 2A, Table 3). Inspection of the contrast parameter estimates revealed that there was simultaneous BOLD signal increase in the right posterior insula in the AN group and BOLD signal decrease in the posterior insula in the HC group in this contrast (Fig. 2A). No further significant differences between groups were found in the amygdala, fusiform gyri, or lateral PFC in this contrast.

Additionally, within the AN group, the mean BOLD signal change in the insula ROI did not significantly correlate with medication status, duration of illness, psychopathology, or BMI in this contrast (Supplementary Table 1).

3.3.2. Fearful > neutral faces

The ROI findings revealed a significant difference between the AN and HC groups in BOLD signal change in left amygdala in response to fearful relative to neutral faces. Similarly, there was a significant difference between groups in BOLD signal change in the left VLPFC in this contrast. Inspection of the peak parameter estimates revealed that there was a relative increase BOLD signal in the left amygdala and VLPFC in response to the fearful expressions in the HC group, but not in the AN group (Fig. 2B,C). No further significant differences between groups were found in the insula or fusiform gyri in this contrast.

Additionally, within the AN group, the mean BOLD signal change in the VLPFC or amygdala ROIs did not significantly correlate with medication status, duration of illness, psychopathology, or BMI in this contrast (Supplementary Table 1).

3.4. Whole brain findings

The exploratory whole brain analysis did not reveal any regions of significant differences in BOLD signal change between groups within the happy > neutral or fearful > neutral contrasts. The effect size maps revealed regions of activation with large effect sizes in response to fearful > neutral and happy > neutral faces in the AN > HC group contrast (Supplementary Fig. 1, Supplementary Fig. 2). However, these regions did not survive whole brain voxel level correction.

4. Discussion

The aim of the current study was to investigate anomalies in the neural substrates employed during implicit processing of positive and negative emotional facial expressions in AN. There was a significant difference between groups in the recruitment of left VLPFC and the amygdala while processing fearful facial expressions, with the HCs showing significantly increased activation in both of these regions while the participants with AN did not. Additionally, we found a significantly increased activation of the right posterior insula in the AN group relative to the HC group while viewing happy facial expressions. No group differences were found in the fusiform gyri in response to fearful or happy facial expressions. Similarly, no group differences were found in the exploratory whole brain analysis.

The current findings revealed reduced activation of the left amygdala in the people with AN relative to the HCs while processing fearful facial expressions. Regarding the HCs, these findings are in line with previous

Table 2
Gender identification mean reaction times and accuracy.

	Trial	AN (N = 20) Mean (SD)	HC (N = 20) Mean (SD)	X^2 statistic, p value
Reaction time (ms)	Fearful faces	934.13 (208.12)	812.78 (143.66)	Trial: $X^2 = 2.09$, p = 0.352
	Happy faces	956.15 (227.28)	827.10 (122.32)	Group: $X^2 = 64.94$, p < 0.001
	Neutral faces	951.20 (204.50)	783.80 (100.98)	Trial x Group: $X^2 = 2.11$, p = 0.348
Accuracy (%)	Fearful faces	85.50 (5.60)	89.00 (5.76)	Trial: $X^2 = 10.15$, p = 0.006
	Happy faces	89.75 (3.02)	88.75 (3.58)	Group: $X^2 = 4.10$, p = 0.043
	Neutral faces	87.13 (4.08)	88.38 (2.84)	Trial x Group: $X^2 = 10.56$, p = 0.005

AN = anorexia nervosa; HC = healthy comparison; ms = milliseconds.

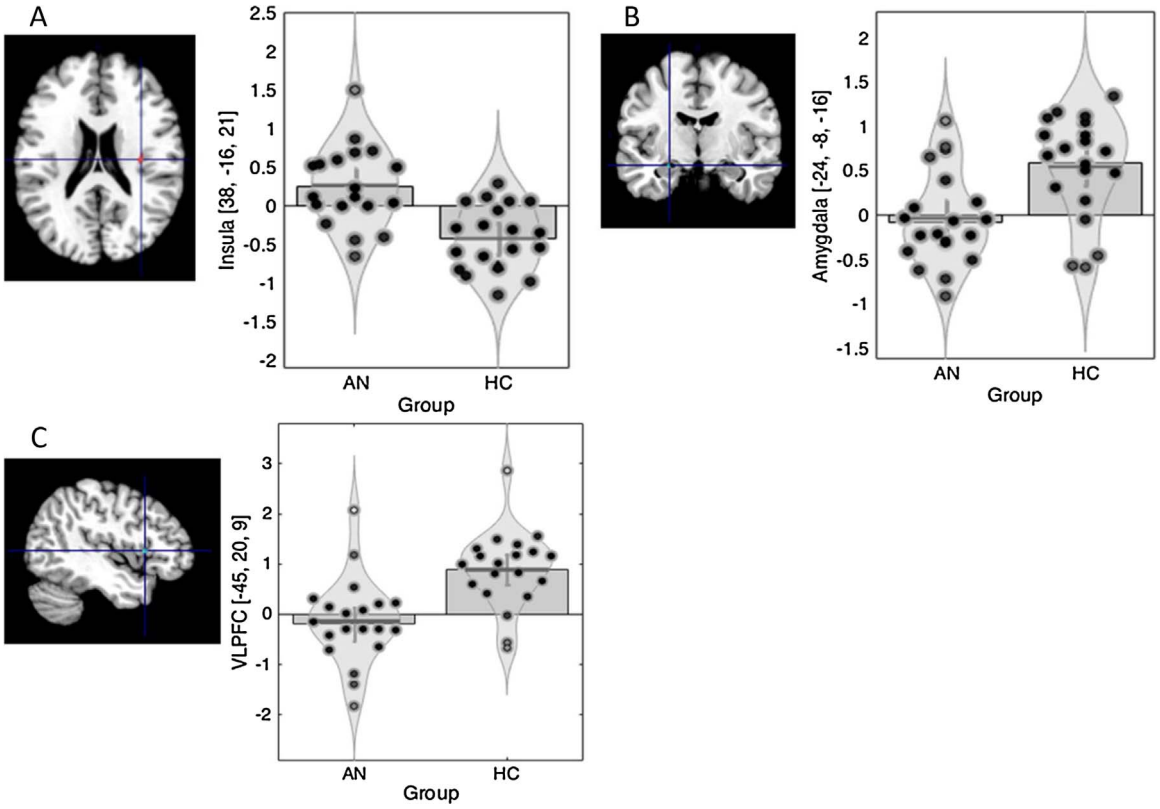

Fig. 2. Regions of interest findings in AN and HC participants.
A.) Differences in signal change between the AN and HC participants in the right posterior insula in the happy > neutral contrast; B.) Differences in signal change between the AN and HC participants in the left amygdala in the fear > neutral contrast; C.) Differences in signal change between the AN and HC participants in the left VLPFC in the fear > neutral contrast. The bar plots indicate group means and the error bars represent the 95% confidence intervals for the mean. The overlaid violin plots provide an indication of the density and distribution of the data and the circles inside the violin plots indicate weighted individual data points. Black circles indicate weight of 1, white circles indicate weight of 0, and grey circles indicate reduced weights. AN = anorexia nervosa; HC = healthy comparison; VLPFC = ventrolateral prefrontal cortex.

work showing that healthy individuals show an elevated amygdala response to negative emotional facial expressions during both implicit and explicit tasks (Fitzgerald, Angstadt, Jelsone, Nathan, & Phan, 2006; Hung et al., 2010; Sato, Yoshikawa, Kochiyama, & Matsumura, 2004). Additionally, previous studies in AN have reported reduced recruitment of the amygdala in people who had recovered from AN relative to HC participants in an explicit facial emotion processing task (Bang et al., 2016). Thus, these findings suggest that blunted amygdala response to negative facial affect in AN may be trait-like anomaly that persist after recovery.

The current findings also showed reduced recruitment of the left VLPFC in participants with AN relative to the HCs, in response to fearful facial expressions. Increased activation of the VLPFC in healthy individuals has been found in studies where participants are presented with negative facial expression or neutral facial expressions in negative context (Kim et al., 2004; Marumo, Takizawa, Kawakubo, Onitsuka, & Kasai, 2009; Taylor, Eisenberger, Saxbe, Lehman, & Lieberman, 2006). In AN, reduced frontal response to emotional facial expression has been previously reported in an EEG study, which found reduced frontal P300, while viewing negative facial expressions (Pollatos, Herbert, Schandry, & Gramann, 2008). Additionally, reduced activation of lateral and medial PFC regions has been reported in AN while viewing positive and negative social behaviour (McAdams & Smith, 2015). Such reduced PFC reactivity has also been associated with poorer general clinical outcome at 1-year follow-up (Schulte-Rüther et al., 2012). These findings highlight the importance of atypical activation of these regions in acute AN and suggest that this could contribute to illness maintenance.

A possible interpretation of these findings is that unlike in depression and anxiety disorders (Groenewold et al., 2013; Mochcovitch et al., 2014), people with acute AN may show generally blunted reactivity to negative social-emotional stimuli. This interpretation is supported by behavioural work that has found that relative to HCs, people with acute AN display less negative facial affect while viewing negative film stimuli (Davies, Schmidt, & Tchanturia, 2013; Davies et al., 2016). People with AN also tend react in an emotionally cold and detached manner to negative emotionally provoking stimuli relative to HCs (Ambwani et al.,

Table 3
Regions of interest findings in AN and HC participants.

Contrast	Peak MNI Coordinates			Contrast signal change					
	x	y	z	AN Mean (SE)	HC Mean (SE)	Max T	*p* value	k	ROI
Happy > Neutral	38	−16	21	0.26 (0.12)	−0.42 (0.10)	4.68	0.0001	14	Insula
Fearful > Neutral	−24	−8	−16	−0.08 (0.13)	0.60 (0.14)	−3.55	0.001	10	Amygdala
	−45	20	9	−0.19 (0.17)	0.90 (0.15)	−4.21	< 0.0001	16	VLPFC

Voxel size 1.5 mm × 1.5 mm × 1.5 mm. AN = anorexia nervosa; HC = healthy comparison; MNI = Montreal Neurological Institute; SE = standard error of the mean; k = number of voxels; ROI = region of interest; VLPFC = ventrolateral prefrontal cortex.

2015). Taken together these finding suggest that people with AN may have atypical blunted response to negative social-emotional cues.

The present study also found a significant increased activation of the right posterior insula in the AN group relative to the HCs while viewing happy facial expressions. A recent meta-analysis found increased activation of the posterior insula in people with AN relative to HC participants when processing stimuli depicting bodies using a variety of paradigms, including comparing own body to images of slim bodies (Zhu et al., 2012). Among healthy individuals, such increased activation of the bilateral posterior insula has previously been associated with perception and experience of emotions, particularly negative emotions (Duerden, Arsalidou, Lee, & Taylor, 2013; Grecucci, Giorgetta, van't Wout, Bonini, & Sanfey, 2013; Waugh et al., 2016). Furthermore, healthy individuals show increased activation of the posterior insula in response to a task involving up-regulation of others' negative intentions (Grecucci et al., 2013).

The above findings suggest that people with AN may exhibit atypical reactivity to positive social-emotional stimuli that is similar to that seen in healthy individuals in response to negative stimuli. This interpretation is supported by previous behavioural work showing that people with AN have a tendency to avoid positive emotions (Lampard, Byrne, McLean, & Fursland, 2011). Additionally, when presented with positive film clips people with AN display fewer reciprocal positive facial expressions, and report less positive subjective positive affect and more negative positive affect than HC participants (Cardi et al., 2015; Davies et al., 2011; Davies et al., 2016; Lang et al., 2016). Additionally, people with AN report greater social anhedonia and general difficulties deriving pleasure from social interactions (Tchanturia et al., 2012). Taken together, these findings suggest that people with AN may have an atypical reaction to positive social-emotional stimuli.

4.1. Clinical implications

The present findings differ from what is seen in depression and anxiety disorders, and suggest that a generally blunted response to negative and atypical response to positive social-emotional information in acute AN. When taken together with recent findings in people who have recovered from AN (Bang et al., 2016), the findings suggest that a blunted response to negatively valenced stimuli may persist after recovery. Reduced reactivity to emotional stimuli has been suggested to have negative affective and social consequences, including elevated negative mood, negative social evaluation by others, and increased isolation (Butler, Lee, & Gross, 2009; Gross, 2002; Szczurek et al., 2012). Furthermore, atypical emotional reactivity in AN also has negative impact on carers and has been associated with increased anxiety and anger in the family (Treasure et al., 2012). Thus, these social-emotional processing difficulties in AN are important targets for interventions.

4.2. Limitations

The main limitation of the current study was the relatively small sample size limiting the exploration of potential confounding effects of AN subtypes, inpatient treatment, and medication. However, we took steps to combat this by exploring the effects of eating disorder psychopathology and medication status on the present findings in post-hoc correlational analyses. Additionally, since we did not include weight restored AN participants or people recovered from AN, it is also not possible to ascertain to what extent the present findings are due to malnutrition. Therefore, future studies with larger and balanced samples are required to replicate and further explore the impact of medication on neural correlates of social-emotional processing in AN. Future, studies would also benefit from further exploring the neural correlates of social-emotional processing difficulties across age and duration of illness.

Although the present study explored the impact of self-reported eating pathology and medication status on the findings in correlational analyses, we did not examine the impact of comorbid disorders. Previous studies have reported that people with AN report higher levels of a number of comorbidities that could impact social-emotional processing, such as autistic symptoms and alexithymia (Caglar-Nazali et al., 2014; Doris, Westwood, Mandy, & Tchanturia, 2014; Westwood et al., 2016).Future studies would benefit from investigating the impact of autistic symptoms and alexithymia on neural processes recruited during social processing in AN.

Finally, the current study only used happy and fearful facial expressions. Utilising a wider range of emotional facial expressions, including disgust, anger and sadness would be of interest. Furthermore, the current study also only used static images without context. Future studies would benefit from using more ecologically relevant designs that include dynamic facial expressions with contextual information.

5. Conclusions

The current study investigated neural mechanisms that underlie processing of fearful and happy facial expressions in AN. The ROI findings revealed decreased BOLD signal in the left amygdala and VLPFC while processing fearful facial expressions in the AN participants compared to HC. Additionally, we found a cluster in the right posterior insula with a relative increase in the BOLD signal while processing happy facial expressions in AN, but not in the HC participants. These findings go some way to support the social-emotional elements of the cognitive-interpersonal maintenance model and calls for interventions that target difficulties in social-emotional difficulties in AN.

Conflict of interest

None

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biopsycho.2017.07.010.

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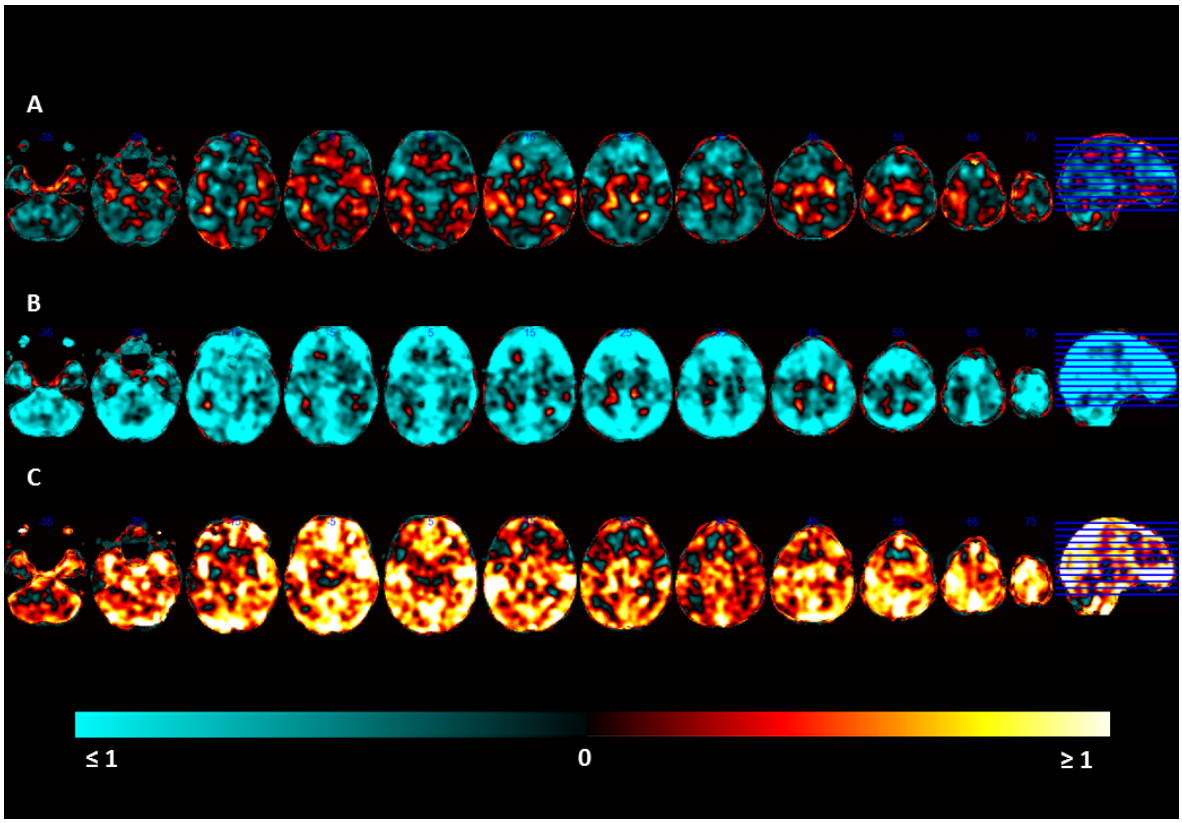
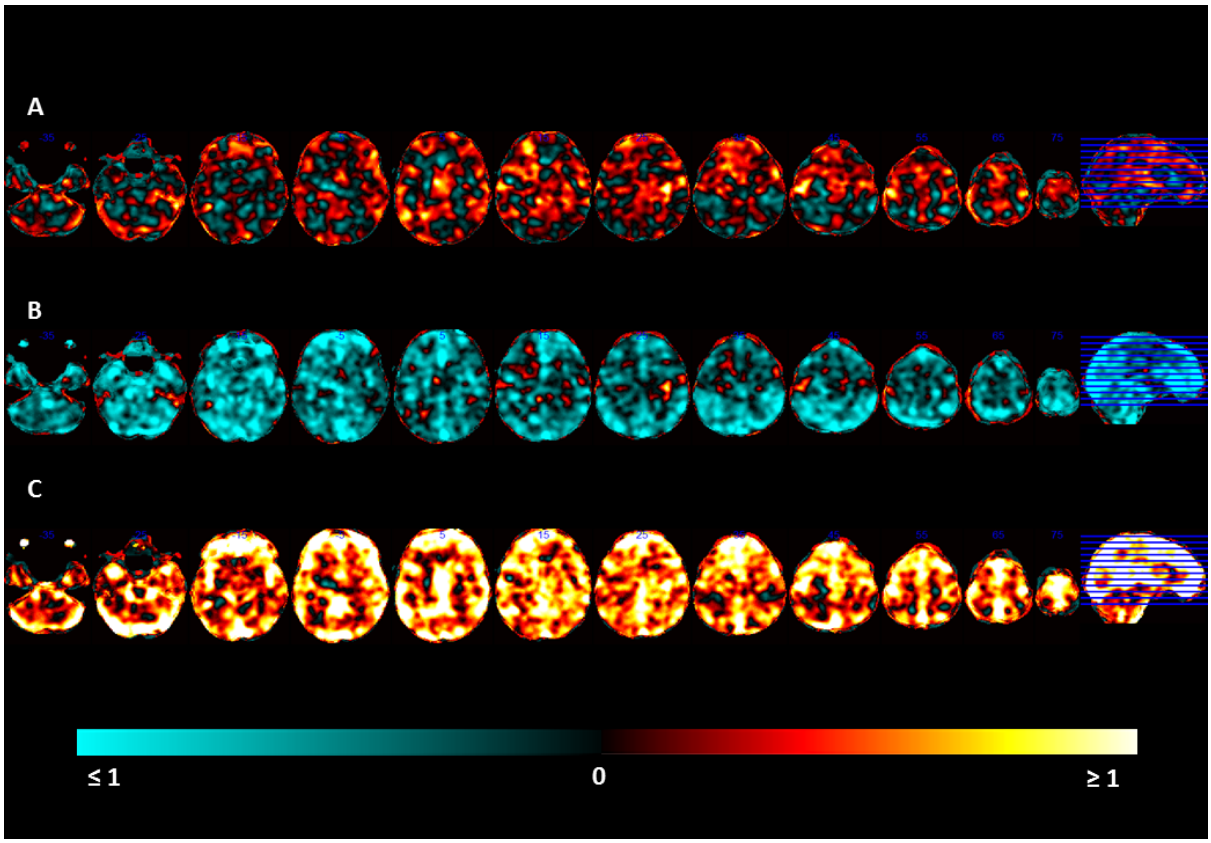
Supplementary Figure 1. Effect size and confidence interval maps of the fear > neutral faces contrast



A.) Hedges' g effect size map of the fear > neutral faces contrast. Positive effect size indicates increased BOLD signal change in the AN participants relative to the HC participants (red to white colours). Negative effect size indicates increased BOLD signal change in the HC participants relative to the AN participants (dark to lighter blue colours). B.) Lower bound of the 99.9% confidence interval lower bound. C.) Upper bound of the 99.9% confidence interval.

Supplementary Figure 2. Effect size and confidence interval maps of the happy > neutral faces contrast



A.) Hedges' g effect size map of the happy > neutral faces contrast. Positive effect size indicates increased BOLD signal change in the AN participants relative to the HC participants (red to white colours). Negative effect size indicates increased BOLD signal change in the HC participants relative to the AN participants (dark to lighter blue colours). B.) Lower bound of the 99.9% confidence interval lower bound. C.) Upper bound of the 99.9% confidence interval.

Supplementary Table 1. Correlations between mean signal change and medication status, BMI, and psychopathology among the AN participants

Contrast	Region	Medication status	EDEQ total	DASS total	BMI	Duration of illness
Happy > Neutral	Insula	ρ = -0.16, p = 0.512	ρ = 0.25, p = 0.306	ρ = -0.01, p = 0.976	ρ = 0.28, p = 0.237	ρ = -0.48, p = 0.031
Fear > Neutral	Amygdala	ρ = 0.11, p = 0.659	ρ = 0.01, p = 0.981	ρ = -0.06, p = 0.823	ρ = 0.22, p = 0.357	ρ = -0.04, p = 0.866
	VLPFC	ρ = -0.05, p = 0.837	ρ = 0.18, p = 0.467	ρ = 0.29, p = 0.228	ρ = 0.02, p = 0.941	ρ = 0.43, p = 0.058

EDEQ = Eating Disorder Examination Questionnaire; DASS = Depression, Anxiety, and Stress Scale; BMI = body mass index; DVLPFC = ventrolateral prefrontal cortex

CHAPTER 4:

FMRI Study of Neural Responses to Implicit Infant Emotion in Anorexia Nervosa

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FMRI Study of Neural Responses to Implicit Infant Emotion in Anorexia Nervosa

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Difficulties in social–emotional processing have been proposed to play an important role in the development and maintenance of anorexia nervosa (AN). Few studies, thus far, have investigated neural processes that underlie these difficulties, including processing emotional facial expressions. However, the majority of these studies have investigated neural responses to adult emotional display, which may be confounded by elevated sensitivity to social rank and threat in AN. Therefore, the aim of this study was to investigate the neural processes underlying implicit processing of positively and negatively valenced infant emotional display in AN. Twenty-one adult women with AN and twenty-six healthy comparison (HC) women were presented with images of positively valenced, negatively valenced, and neutral infant faces during a fMRI scan. Significant differences between the groups in positive > neutral and negative > neutral contrasts were investigated in *a priori* regions of interest, including the bilateral amygdala, insula, and lateral prefrontal cortex (PFC). The findings revealed that the AN participants showed relatively increased recruitment while the HC participants showed relatively reduced recruitment of the bilateral amygdala and the right dorsolateral PFC in the positive > neutral contrast. In the negative > neutral contrast, the AN group showed relatively increased recruitment of the left posterior insula while the HC groups showed relatively reduced recruitment of this region. These findings suggest that people with AN may engage in implicit prefrontal down-regulation of elevated limbic reactivity to positively social–emotional stimuli.

Keywords: eating disorders, anorexia nervosa, fMRI, emotional infant faces, implicit

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INTRODUCTION

Recent large scale meta-analyses and systematic reviews have found that people with anorexia nervosa (AN) have difficulties in various aspects of social–emotional processing, including theory of mind and accurate interpretation of emotions (Caglar-Nazali et al., 2014; Bora and Köse, 2016). Another recent meta-analysis also found that people with AN show a behavioral pattern of reduced expression of emotions when viewing both positive and negative emotionally provoking stimuli (Davies et al., 2016). Additionally, behavioral studies have reported that people with AN also show greater attentional bias toward threatening emotional facial expressions, such as rejecting

and angry faces, and attentional avoidance of positive facial expressions, such as accepting and happy faces (Harrison et al., 2010; Cardi et al., 2013, 2015). Furthermore, people with AN show elevated sensitivity to social rank, and report more submissive behavior and feelings of shame (Troop and Baker, 2008; Cardi et al., 2014b; Troop, 2016). These difficulties have been proposed to play an important role in the development and maintenance of disordered eating in AN, increasing isolation and negative mood (Treasure and Schmidt, 2013). Further investigation of the underlying mechanisms is of interest.

Few studies to date have investigated the neural mechanisms that underlie these difficulties in social–emotional processing in AN. A recent systematic review of the literature found that relative to healthy comparison (HC) participants, people with AN show atypical, reduced recruitment in regions such as lateral and medial prefrontal cortex (PFC) in response to social–emotional behavior (McAdams and Smith, 2015). Additionally, a prospective study by Schulte-Rüther et al. (2012) found that reduced recruitment of the prefrontal regions in response to social–emotional behavior at admission to hospital was associated with poorer outcome. Interestingly, other studies have found that when asked to select negatively valenced social words or view provoking stimuli, such as food images, people with AN show greater recruitment of cortical regions, including the dorsolateral PFC and insula, relative to healthy control (HC) participants (Brooks et al., 2011, 2012a; Miyake et al., 2012; Brooks, 2016). Additionally, a recent study by Fonville et al. (2014) investigated neural response to positive facial expressions in acute AN and found a linear increase in neural activation in the right fusiform gyrus, which was greater in the AN group than the HC group. Taken together, these findings suggest that atypical recruitment of lateral prefrontal, insular, and visual attention regions may underlie difficulties in social–emotional processing in AN.

The above mentioned studies have largely focused on investigating atypical emotional processing in the context of peer, adult social–emotional display, which may be confounded by elevated sensitivity to social rank and threat in AN. It would be of interest to explore whether atypical emotional processing extends across lifespan to, for example pre-language infant emotional display. Adults in general are uniquely attuned to social–emotional signaling from infant faces due to absence of language (Brosch et al., 2007; Parsons et al., 2011; Thompson-Booth et al., 2014). Additionally, relative to adult faces infant faces have been found to be perceived as less threatening, more helpless, and evoke caregiving responses in adults (Berry and McArthur, 1985, 1986; Parsons et al., 2011; Senese et al., 2013). Neuroimaging studies have found that relative to adult faces infant faces strongly activate regions involved in social–emotional processing including the lateral PFC and insula, and regions involved in visual attention, such the fusiform gyrus (Caria et al., 2012; Rocchetti et al., 2014). Furthermore, emotional infant faces have been found to strongly recruit limbic regions including the amygdala, as well as multiple areas in the frontal cortex, including the lateral PFC, in healthy adults (Baeken et al., 2009; Montoya et al., 2012).

A study by Cardi et al. (2014a) found that people with eating disorders (EDs) show anomalies in processing infant emotional display. Interestingly, unlike with emotional adult faces, Cardi et al. (2014a) found no significant differences between the people with EDs and HC participants in attentional bias toward positive and negatively valenced emotional infant faces (Cardi et al., 2015). However, relative to HC participants, people with EDs interpreted the positively valenced infant stimuli to be less positive and reported more subjective negative affect in response to negative infant display (Cardi et al., 2014a). Additionally, the participants with EDs displayed fewer positive facial expressions while viewing a positively valenced infant display (Cardi et al., 2014a). These findings suggest that people with EDs may have a tendency to interpret emotional stimuli in a negative way and display reduced facial affect, which are not restricted to peer, other-adult emotional displays, but extends to less threatening and motivationally salient infant emotional expression. However, to our knowledge no studies to date have investigated the neural responses to infant emotional expression in EDs, which could shed light on the mechanisms that may underlie these atypical responses.

Few studies in mood and anxiety disorders, common co-morbid disorders in AN, have investigated neural responses to motivationally salient emotional infant stimuli (Baeken et al., 2010; Schechter et al., 2012; Wonch et al., 2016). A study investigating neural responses to emotional infant stimuli among people with melancholic depression found increased activation in regions including the ventrolateral PFC (VLPFC) and inferior occipital cortex, while viewing positively valenced infant faces relative to scrambled stimuli (Baeken et al., 2010). Another study found that relative to healthy mothers, mothers with postnatal depression showed greater amygdala response to unfamiliar positively valenced infant faces (Wonch et al., 2016). The authors also found that mothers with postnatal depression showed reduced functional connectivity between the amygdala and insula while viewing positively valenced infant stimuli (Wonch et al., 2016). Additionally, previous work has found that mothers with interpersonal trauma related post-traumatic stress disorder (PTSD) showed increased recruitment of regions involved in emotion processing and regulation, including the posterior insula, amygdala, and dorsolateral PFC (DLPFC), while viewing distressed unfamiliar infants relative to content infants (Schechter et al., 2012). Taken together, these findings suggest that there may be deficits in elevated insular and limbic reactivity, and prefrontal down-regulation in response to motivationally salient infant emotion in these disorders.

The aim of the current study was to investigate the neural correlates that underlie implicit processing of infant emotion in people with AN relative to HC participants. Based on the previous findings from studies investigating neural response to social–emotional stimuli in people with AN outlined above, we hypothesized that we would find atypical increased recruitment of regions involved in emotion down-regulation, namely the bilateral lateral PFC, in response to emotional infant faces. Additionally, based on previous neuroimaging work among people with anxiety and mood disorders we hypothesized that the AN group would show atypical elevated neural response

to emotional infant faces in regions associated with emotional processing, including the bilateral amygdala and insula. These hypotheses were investigated with regions of interest approach.

MATERIALS AND METHODS

Participants

Forty-seven adult women took part in the study. Twenty-one women had a current DSM-5 diagnosis of AN. Fifteen women with AN were recruited through advertisements placed on EDs charities websites (BEAT and Succeed) and six women with AN were recruited from the South London and Maudsley NHS Foundation Trust inpatient unit. All AN participants recruited from the inpatient unit were receiving treatment at the time of the study. Twenty-six HCs with a BMI between 18.5 and 25 and no history of psychiatric disorders were recruited amongst King's College London students and staff who responded to advertisements placed on the university's website. The Structured Clinical Interview for DSM-5 was used to confirm AN diagnosis, and to screen for psychiatric disorders in the HC group (First et al., 2015). Both groups were matched for age and level of education. Participants were excluded from the study if they were left handed or reported a history of head trauma, neurological disease, uncorrected hearing or visual impairment, acute suicidality, history of or current alcohol or drug abuse, or MRI incompatibility (i.e., implanted medical devices of any kind, history of accidents involving metal, any metal in or on the body that cannot be removed, claustrophobia, pregnancy). Additional exclusion criteria for the AN group included psychotropic medication other than selective serotonin reuptake inhibitors (SSRIs). Prior to taking part in the study, all participants were asked to give written informed consent, and were compensated for their time. The study was conducted in accordance with the latest version of the Declaration of Helsinki (1975, as revised in 2008) and was approved by a local National Research Ethics Service (NRES) committee (11/LO/0373).

Clinical and Questionnaire Measures

The Eating Disorder Examination Questionnaire (EDEQ), a 36-item self-report measure, was used to assess ED behaviors and attitudes over the past 28 days (Fairburn and Beglin, 1994). In the current study, internal consistency of EDEQ was high, with Cronbach's alpha of 0.90.

The Depression, Anxiety, and Stress scale (DASS) is a 21-item self-report measure assessing severity of depression, anxiety, and stress over the past week (Lovibond and Lovibond, 1995). In the current study, internal consistency of the DASS was high, with Cronbach's alpha of 0.97.

Functional Magnetic Resonance Imaging (fMRI) Procedure

Participants were presented with black and white photographs of prototypical positively valenced (smiling), prototypical negatively valenced (crying), and neutral infant faces during a 12-min fMRI scanning session. The images were matched for physical properties, including size, contrast, and luminosity. The stimuli were acquired from a validated set of infant emotional facial expressions and composed of ten different babies (five female, five male) (Kringelbach et al., 2008). The images were used with approval from the authors.

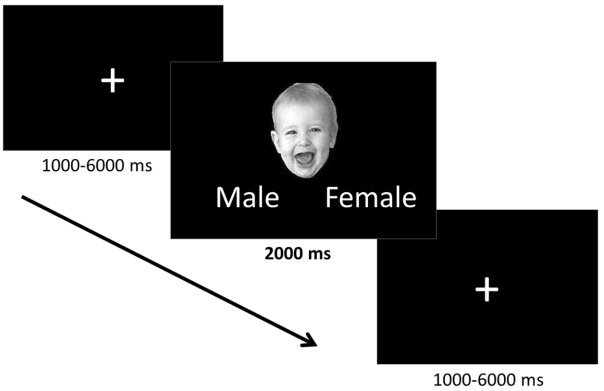

FIGURE 1 | Implicit emotion processing task. The positively valenced, negatively valenced, and neutral infant faces were presented one at a time for 2000 ms during which time participants identified the gender of each face. Faces were preceded by a fixation cross the presentation of which was jittered between 1000 and 6000 ms.

The task employed an event-related design, in which the faces were presented one at a time for 2000 ms separated by a fixation cross (**Figure 1**). Ten positively valenced, ten negatively valenced, and ten neutral infant faces were presented in a pseudorandomized order to avoid learning effects and avoid positive and negative faces following one another. The fixation cross inter-stimulus interval was jittered to vary between 1 and 6 s (mean 3.0 s) in order to prevent participants from being able to predict the onset of the trials. While viewing the faces participants were asked to indicate the gender of the faces using the control pad to ensure that they paid attention to the stimuli. The participants were told that the gender identification task would be difficult, to not think about their answers too much, and give their best guess. They were informed that the main goal was to attend to the stimuli and were not given feedback regarding their performance during the task.

Data Acquisition

The GE Signa 1.5 Tesla scanner (GE Medical Systems, Milwaukee, WI, USA) was used to acquire the magnetic resonance images at the King's College London, Centre for Neuroimaging Sciences (CNS). An 8-channel radiofrequency birdcage head coil was used to transmit and receive the signal. High resolution T1-weighted magnetization-prepared rapid gradient echo (MP-RAGE) structural images were acquired with a repetition time (TR) of 8.592 ms, and 1.2 mm slice gap, and 1.2 mm slice thickness. One hundred and eighty slices were used to achieve whole brain coverage with an in-plane resolution of 1.25 mm × 1.25 mm. Following the structural scan, a functional blood oxygen level dependent (BOLD) signal was acquired using interleaved T2* weighted echo planar imaging (EPI) with TR

of 2 s, and 0.4 mm slice gap, and 4 mm slice thickness. Thirty slices were used to achieve whole brain coverage with in-plane resolution of 3.75 mm × 3.75 mm. Data quality was assured using an automated quality control procedure (Simmons et al., 1999).

Statistical Analysis

Behavioral and Self-report Data

Behavioral and self-report questionnaire data were analyzed with Stata 14 (StataCorp. 2015, College Station, TX, USA: StataCorp LP.). Group differences in demographic variables and questionnaire responses were investigated using non-parametric median Chi^2 tests. The behavioral data from the gender identification task was analyzed with 2 × 3 linear mixed models with group (AN, HC) and valence (positive, negative, neutral) entered as fixed effects and a random intercept. Significant interactions were explored with *post hoc* contrasts and pairwise comparisons. *P*-values of 0.05 or lower were considered significant.

Functional Neuroimaging Data

FMRI data pre-processing was conducted using SPM8 (Wellcome Department of Cognitive Neurology, Institute of Neurology, London) implemented in MATLAB, version 8.2.0 (Mathworks, Natick, MA, USA). The functional data was corrected for slice-timing and volume-to-volume head motion. The functional data was re-sliced and following re-slicing the voxel size was 1.5 mm × 1.5 mm × 1.5 mm. Following correction the functional images were co-registered to a T1-weighted DARTEL template created from each participant's high resolution MP-RAGE structural image (Ashburner, 2007). The images were smoothed with an 8 mm FWHM three-dimensional isotropic Gaussian Kernel and normalized to Montreal Neurological institute (MNI) space.

At subject level, the data was modeled using the general linear model framework implemented in SPM8. The BOLD signal was modeled using a single canonical HRF, and the predicted BOLD response was modeled for each of the following conditions: positively valenced faces, negative valenced faces, and neutral faces. Twenty four motion parameters were calculated and used to adjust the time series data for head motion (Friston et al., 1996). Low frequency drift was filtered out of the data using a high-pass filter set to 1/128 s. The subject level model produced the following contrast images: positive faces > neutral faces, and negative faces > neutral faces.

The robust regression toolbox (Wager et al., 2005)[1] implemented in Matlab 2016b was used to conduct group level random effects analysis. The robust regression toolbox uses iteratively re-weighted least squares (IRLS), which identifies and outweighs influential, extreme outliers. Thus, the IRLS analysis minimizes the impact of extreme outliers and reduces the likelihood of false positive and negative findings with no reduction in power (Wager et al., 2005; Fritsch et al., 2015). We chose this approach to avoid false findings arising from head motion, scanner-related artifacts, or individual participants who were particularly depressed, anxious, stressed, or otherwise vastly differed from the rest of the sample during the MRI scan, which could have led them to process the social–emotional stimuli in a different way (Leppänen et al., 2004; Wager et al., 2005; Kircanski et al., 2014).

The contrast images were first entered into region of interest (ROI) analyses to investigate *a priori* hypothesis within the following bilateral regions: amygdala, insula, and lateral PFC. Anatomical masks of these regions were created using the WFU Pickatlas implemented in SPM8. In the IRLS analysis group was added as a contrast coded covariate (1, −1: AN, HC) with positive test statistics indicating relatively increased activation in the AN group and negative test statistics indicating relatively increased activation in the HC group. The ROI findings were corrected for multiple comparisons with a voxel-wise non-parametric permutation test as recommended by Eklund et al. (2016) ($\alpha < 0.05$). The permutation test uses the max T distribution to identity a critical threshold to control for family-wise error rate. The permutation test was conducted with 10000 iterations.

Where significant group differences were present, mean signal change data was extracted from the clusters. The data was entered into further analysis to investigate if the atypical activation within the AN group correlated with BMI, duration of illness, medication status, DASS total score, or EDEQ total score. The correlational analyses were conducted using Spearman's rho in Matlab 2016b.

Whole brain exploratory analysis was conducted to investigate brain responses to positive and negative infant faces in AN. As above, in order to explore differences in brain responses between AN and HC groups, group was added as a contrast coded covariate (1, −1: AN, HC) in the IRLS analyses. The following contrasts were included in the whole brain analysis: positive faces > neutral faces and negative faces > neutral faces. The whole brain analysis was corrected with voxel-wise False Discovery Rate (FDR) thresholded at $q < 0.05$. Effect size, and lower and upper bound 99.9% confidence interval maps for the whole brain analyses were generated using the EScalc toolbox implemented in Matlab[2] (Gao and Zang, 2015), and are presented in **Supplementary Figure S1** (Positive > Neutral) and **Supplementary Figure S2** (Negative > Neutral).

RESULTS

Group Characteristics

Demographic and clinical characteristics of both groups are presented in **Table 1**. Participants did not differ significantly in age or level of education. However, as expected, the AN group had significantly lower BMI and reported higher depression, anxiety, stress, and ED psychopathology than the HC group. Medication status provides the number and the percentage of AN participants taking SSRIs during the time of the study.

[1] https://github.com/canlab/RobustToolbox

[2] http://restfmri.net

TABLE 1 | Sample clinical and demographic characteristics.

	AN ($N = 21$) Median [Q1, Q3]	HC ($N = 26$) Median [Q1, Q3]	X^2 (DF) statistic, p-value
Age	25.00 [20.00, 34.50]	25.50 [23.00, 28.00]	X^2 (1) = 0.001, $p = 0.969$
Level of education (years)	16.00 [14.00, 17.00]	17.00 [14.00, 20.00]	X^2 (1) = 0.09, $p = 0.766$
BMI	15.84 [14.8, 16.76]	19.90 [19.38, 21.88]	X^2 (1) = 24.68, $p < 0.001$
Medication status *N* (%)	12 (57%)	–	–
EDEQ total	3.87 [3.41, 5.02]	0.31 [0.14, 0.58]	X^2 (1) = 34.04, $p < 0.001$
EDEQ restraint	4.20 [3.60, 5.20]	0.20 [0.00, 0.60]	X^2 (1) = 30.39, $p < 0.001$
EDEQ eating concern	3.90 [3.20, 4.40]	0.00 [0.00, 0.20]	X^2 (1) = 37.38, $p < 0.001$
EDEQ weight concern	3.80 [3.00, 5.50]	0.40 [0.00, 0.60]	X^2 (1) = 27.40, $p < 0.001$
EDEQ Shape concern	4.75 [3.44, 5.69]	0.63 [0.25, 1.13]	X^2 (1) = 37.38, $p < 0.001$
DASS total	67.00 [54.00, 88.00]	9.00 [4.00, 12.00]	X^2 (1) = 31.93, $p < 0.001$
DASS anxiety	19.00 [13.00, 23.00]	0.00 [0.00, 2.00]	X^2 (1) = 25.57, $p < 0.001$
DASS depression	24.00 [18.00, 35.00]	2.00 [0.00, 4.00]	X^2 (1) = 25.57, $p < 0.001$
DASS stress	28.00 [19.00, 34.00]	4.00 [2.00, 10.00]	X^2 (1) = 25.57, $p < 0.001$

AN, anorexia nervosa; HC, healthy comparison; BMI, body mass index; EDEQ, Eating Disorder Examination Questionnaire; DASS, Depression, Anxiety, and Stress Scale; Medication status, percentage of AN participants taking anti-depressants; Q1, first quartile, Q3, third quartile, DF, degrees of freedom.

Task Performance

The gender identification task performance in the AN and HC groups is presented in **Table 2**. Regarding accuracy, the mixed effects model revealed a significant effect of trial with participants performing better in the neutral trials than positive trials ($Z = 24.97$, $p < 0.001$, 95% CI [0.43, 0.50]) or negative trials ($Z = 24.32$, $p < 0.001$, 95% CI [0.44, 0.52]). There were no significant differences between participants performance on the positive and negative trials ($Z = -0.95$, $p = 0.340$, 95% CI [−0.06, 0.02]).

Similarly, the mixed effects model also revealed a significant effect of trial in participants' reaction times (**Table 2**). Participants were significantly faster in the neutral trials than positive trials ($Z = -3.59$, $p < 0.001$, 95% CI [−125.03, −36.75]) and positive trials than negative trials ($Z = -2.62$, $p = 0.009$, 95% CI [−117.78, −17.01]). There were no significant differences between participants reaction times in the neutral and negative trials ($Z = -0.61$, $p = 0.540$, 95% CI [−56.68, 29.69]). There was also a significant effect of group in participants' reaction times (**Table 2**), with HC participants responding significantly faster than AN participants across trials ($Z = -3.70$, $p < 0.001$, 95% CI [−158.04, −48.55]).

Regions of Interest Findings

We conducted ROI IRLS analyses to investigate group differences in activation in response to positively valenced and negatively valenced infant faces within the following masks: amygdala, insula, and lateral PFC. The ROI findings for group differences are presented in **Figure 2** and **Table 3**.

Positive > Neutral

The ROI findings revealed a significant difference between the groups in recruitment of a very small cluster in the right DLPFC in the positive > neutral contrast (**Table 3**). Exploration of the mean contrast signal change suggested that the group differences in the DLPFC was driven by relatively increase in recruitment in the AN group in this contrast (**Figure 2A**). Similarly, a significant difference between the groups in recruitment of the bilateral amygdala in the positive > neutral contrast was observed (**Table 3**). Exploration of the mean contrast signal change suggested that the group difference observed in the amygdala was driven by relatively increased recruitment in the AN group and reduced recruitment in the HC group in this contrast (**Figures 2B,C**).

Post hoc correlational analysis within the AN group revealed that the mean contrast signal change in the DLPFC and amygdala ROIs did not significantly correlate with psychopathology, medication status, BMI, or duration of illness in this contrast (Supplementary Table S1).

Negative > Neutral

There was a significant difference between the AN and HC groups in recruitment of the left posterior insula in the negative > neutral contrast (**Table 3**). Exploration of the mean contrast signal change revealed that the group difference was brought on by relatively increased recruitment in the AN group and reduced recruitment in the HC group in this contrast (**Figure 2D**).

Post hoc correlational analysis within the AN group revealed that the mean contrast signal change in the insula ROI did not significantly correlate with psychopathology, medication status, BMI, or duration of illness in this contrast (Supplementary Table S1).

Whole Brain Findings

The positively valenced faces > neutral faces and negatively valenced faces > neutral faces contrasts did not yield areas of significant group differences in the whole brain exploratory search.

DISCUSSION

The aim of the current study was to investigate differences in neural mechanisms that underlie implicit processing of

TABLE 2 | Gender identification task performance in the AN and HC groups.

	Trial	AN ($N = 21$)	HC ($N = 26$)	X^2 (DF) statistic, p-value
Accuracy (%)	Positive	27.50 (9.80)	30.19 (8.42)	Group: X^2 (1) = 0.13, $p = 0.717$
	Negative	28.00 (7.68)	27.50 (7.78)	Trial: X^2 (2) = 832.59, $p < 0.001$
	Neutral	73.55 (7.61)	72.73 (5.29)	Group × Trial: X^2 (2) = 0.85, $p = 0.653$
RT (ms)	Positive	1197.72 (433.12)	1094.15 (182.48)	Group: X^2 (1) = 13.68, $p = 0.0002$
	Negative	1254.36 (352.47)	1172.30 (190.39)	Trial: X^2 (2) = 13.29, $p = 0.001$
	Neutral	1194.57 (392.48)	1070.32 (154.87)	Group × Trial: X^2 (2) = 0.94, $p = 0.624$

AN, anorexia nervosa; HC, healthy comparison; RT, reaction time, DF, degrees of freedom.

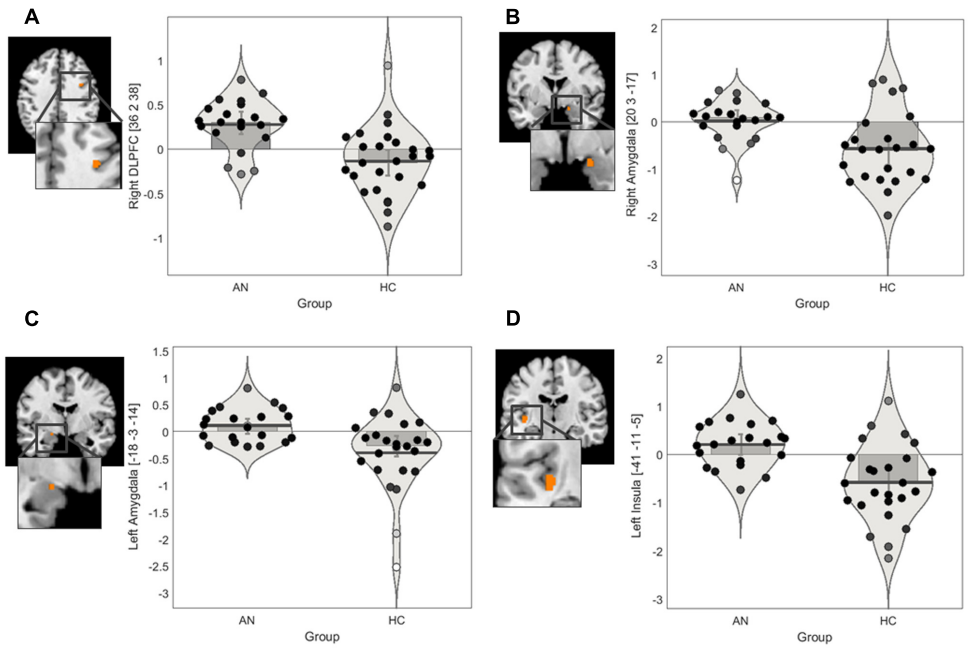

FIGURE 2 | Regions of interest findings in AN and HC participants. (A) Statistical parametric map of the right lateral PFC ROI with parameter estimate bar plot showing mean activation in the right DLPFC cluster in the positive > neutral contrast. **(B)** Statistical parametric map of the bilateral amygdala ROI with parameter estimate bar plot showing mean activation in the right amygdala cluster in the positive > neutral contrast **(C)** Statistical parametric map of the bilateral amygdala ROI with parameter estimate bar plot showing mean activation in the left amygdala cluster in the positive > neutral contrast. **(D)** Statistical parametric map of the bilateral insula ROI with parameter estimate bar plot showing mean activation in the left posterior insula cluster in the negative > neutral contrast. The *Y*-axis shows mean contrast signal change.

emotional infant faces between AN and HC participants. As hypothesized, group differences emerged in the recruitment of the left posterior insula, bilateral amygdala, and the right DLPFC between the AN and HC groups. A very small cluster in the right DLPFC showed increased recruitment in the AN group, but not the HC group, while viewing positively valenced infant

TABLE 3 | Regions of interest findings in AN and HC participants.

	Peak MNI coordinates			Contrast signal change				
Contrast	***x***	***y***	***z***	**AN *Mean* (*SE*)**	**HC *Mean* (*SE*)**	**Max T statistic, *p*-value**	***k***	**ROI**
Positive > Neutral	−18	−3	−14	0.10 (0.07)	−0.27 (0.10)	$t = 3.85$, $p = 0.0008$	24	Amygdala
	20	3	−17	0.09 (0.08)	−0.60 (0.15)	$t = 3.54$, $p = 0.0002$	14	Amygdala
	36	2	38	0.30 (0.06)	−0.15 (0.07)	$t = 4.17$, $p < 0.0001$	4	DLPFC
Negative > Neutral	−41	−11	−5	0.20 (0.11)	−0.58 (0.17)	$t = 4.18$, $p = 0.0001$	24	Insula

AN, anorexia nervosa; HC, healthy comparison; ROI, region of interest; MNI, Montreal Neurological Institute; DLPFC, dorsolateral prefrontal cortex; SE, standard error of mean; k, number of voxels; voxel size 1.5 mm × 1.5 mm × 1.5 mm.

faces relative to neutral faces. Interestingly, the bilateral amygdala showed relatively reduced recruitment in the HC group, but not the AN group, in this contrast. While viewing negatively valenced faces relative to neutral faces, the AN groups showed increased recruitment of the left posterior insula, while the HC group showed relatively reduced recruitment of this region. The whole brain analysis did not reveal any further regions showing significant differences between the groups in either contrast.

The present ROI findings revealed increased recruitment of a very small cluster in the right DLPFC in the AN group relative to the HC group when viewing images of positively valenced infant faces. Similar increased recruitment of the lateral PFC has also been found in women with melancholic depression while viewing positively valenced infant faces (Baeken et al., 2010). Increased recruitment of the right DLPFC has also been found among people with depression while viewing salient, positive emotional stimuli, including smiling faces (Jaworska et al., 2015). Further, one study reported a positive correlation between recruitment of the right DLPFC and valence rating of emotional stimuli among people with depression, whereas an inverse correlation was observed among healthy individuals (Grimm et al., 2008). Indeed, increased recruitment of the right DLPFC is often reported among healthy participants during working memory tasks, and while processing negative emotional stimuli particularly when explicitly asked to down-regulate subjective negative emotional responses to intense distressing stimuli (Goldin et al., 2008; Wager et al., 2008; Ochsner et al., 2012; Criaud and Boulinguez, 2013). Similarly, increased lateral and medial prefrontal activation has been reported among healthy women in response to negatively valenced infant stimuli, including images of distressed infants and the sound of infant cry (Baeken et al., 2009; Montoya et al., 2012). Thus it appears that in the healthy population the DLPFC activation is as associated with a form of top-down control to regulate emotional responses to negative stimuli. However, it seems that in AN as well as depression this mechanism may be activated in response to positive emotional stimuli. Still, it is of importance to note that the cluster in the right DLPFC in the present study was very small and replication of these findings is necessary before firm conclusions can be drawn.

The present study also found relatively reduced recruitment of the bilateral amygdala among HC participants, but not among AN participants, while viewing positively valenced infant faces. Reduced recruitment of the bilateral amygdala has also previously been found among healthy individuals while processing positive emotional adult faces (Cowdrey et al., 2012). Interestingly, similar reduced recruitment of the amygdala in response to positive facial expressions has also been reported in people who have recovered from AN (Cowdrey et al., 2012). Additionally, relatively increased recruitment of the amygdala has been found previously among mothers with postnatal depression while viewing positive infant stimuli (Wonch et al., 2016). Furthermore, a number of studies have found that in addition to increased recruitment of the right DLPFC, people with depression show relatively increased recruitment of the amygdala while processing salient, positive emotional stimuli (Jaworska et al., 2015). One study also reported correlated increase in the recruitment of the bilateral amygdala and right DLPFC in people with depression while processing positive emotional facial expressions (Liao et al., 2012). Moreover, as with people recovered from AN, a few studies investigating differences in neural activation in response to positive social–emotional stimuli between people recovered from depression and HC participants have reported no significant differences in the recruitment of the amygdala (Dutra, 2012; Kerestes et al., 2012). Taken together these findings suggest that atypical activation of the bilateral amygdala while processing salient positive social–emotional stimuli may be related to acute state of illness in AN and depression.

A possible interpretation of the above findings is that people with acute AN may engage in implicit prefrontal down-regulation of elevated amygdala reactivity to positively valenced infant stimuli. Indeed, the wealth of previous work in depression has suggested that increased recruitment of the DLPFC and the amygdala in response to positive emotional stimuli to be linked with attempts to down-regulate positive mood (Kupfer et al., 2012). Previous studies have also found that people with AN show greater implicit cognitive control relative to HC during working memory tasks demonstrated by equivalent behavioral performance, but elevated DLPFC activation (Brooks et al., 2012b; Brooks, 2016). This interpretation would be in line with findings from previous behavioral studies reporting reduced facial expressivity in people with AN while viewing positive infant stimuli (Cardi et al., 2014a). Furthermore, these findings are also supported by the steady accumulation of studies reporting reduced facial expressivity in response to general emotionally provoking positive films among people with AN (Davies et al., 2016). Positive facial expressivity has also been found to correlate positively with BMI and negatively with ED psychopathology among people with acute AN (Dapelo et al., 2016; Lang et al., 2016). People recovered from AN, on the other hand, do not display similar reduced facial expressivity to emotionally provoking positive stimuli (Davies et al., 2013). However, as the cluster in the right DLPFC in the present study was very small, replication of these findings with a larger sample is necessary before firm conclusions can be drawn.

The present findings also revealed relatively increased recruitment of the left posterior insula in the AN group and relatively reduced recruitment of this region in the HC group when viewing negatively valenced infant faces. Similar pattern of increased recruitment of the left posterior insula has been seen in mothers with interpersonal trauma related PTSD while viewing videos of distressed unfamiliar infants (Schechter et al., 2012). The increased recruitment of the posterior insula was also associated with increased levels of subjective distress as reported by these mothers, which has been speculated to indicate emotion dysregulation and difficulties in down-regulating subjective negative emotions such as feelings of distress and helplessness (Schechter et al., 2012). Furthermore, increased recruitment of posterior insula has been previously found in response to intense sadness and distress in healthy and depressed individuals, with recovery from depression being marked by decreased recruitment of the posterior insula (Mayberg et al., 1999).

Thus, a possible interpretation of this finding is that people with AN may experience greater subjective distress while viewing

salient, negative infant stimuli. Indeed, the posterior insula has been suggested to play an important role in processing of emotional salience and interoceptive awareness (Craig, 2009; Menon and Uddin, 2010; Duerden et al., 2013). Hyperactivation of this region in response to negative emotional stimuli has been associated with subjective feelings of distress among healthy and clinical populations (Mayberg et al., 1999; Schechter et al., 2012). This would be in line with behavioral studies showing that people with AN report generally elevated social anxiety and distress, and report more subjective negative affect in response to emotionally provoking negative stimuli (Gilboa-Schechtman et al., 2006; Lang et al., 2016). Furthermore, one study found that subjective distress significantly mediated difficulties in emotional awareness and attention toward emotions in AN (Gilboa-Schechtman et al., 2006). Taken together, these findings suggest that posterior insula may play an important role in processing negative emotional information in AN and further exploration of this regions in context of subjective negative affect would be of interest.

Clinical Implications

The current findings are in line with previous behavioral studies suggesting that people with AN may engage in implicit down-regulation of emotions and report elevated subjective distress while viewing particularly salient positive and negative stimuli, respectively (Cardi et al., 2014a; Davies et al., 2016). Suppression of emotional responses can have generally negative emotional and social consequences, including elevated negative mood and social isolation (Gross, 2002; Szczurek et al., 2012). Furthermore, reduced emotional expression in response to infant emotional display can have profoundly disruptive effect on the infant as demonstrated with the still face paradigm (Weinberg and Tronick, 1994) and this may ultimately impact the development of the children of mothers with AN (Micali et al., 2014). Thus, these findings further highlight the need for interventions that target atypical social–emotional processing, including down-regulation of positive emotions and elevated subjective distress, in AN. Pending replication, the present findings may serve as useful targets to assess effectiveness of such interventions.

Limitations

The main limitation of this study was the gender identification task chosen to ensure that participants paid attention to the stimuli and allow exploration of neural mechanisms underlying implicit emotion processing. Although this task has been previously used successfully with images of adult faces (Fonville et al., 2014), in the present study both groups exhibited poor accuracy in identifying the infants' gender in the emotional context. Tasks that the participants find too difficult can lead to increased recruitment of other regions in order to try and cope with the task demands or, in some cases, participants may "give up" leading to less time spent on task (Bookheimer, 2000; Huddleston and DeYoe, 2008; Pressman and Gitelman, 2012). Additionally, increasing task demands can influence the processing of social–emotional stimuli leading to reduced recruitment of regions typically involved in emotion processing, such as the amygdala (Blair et al., 2007). Although, in the current study both groups performed equally poorly and they were not given feedback regarding their performance, it is not possible to ascertain that the group differences observed were not partly due to the AN participants feeling guilty about their poor performance. Before firm conclusions can be drawn from the present findings, these results must be replicated with an alternative task and future research should use alternative tasks to further investigate implicit emotion processing.

Although the aim of the present study was to investigate the neural mechanisms that underlie implicit processing of infant emotional display, it would have been of interest to also investigate neural processes that underlie explicit recognition of emotions in infant faces. There is behavioral evidence that people with EDs show negative bias when interpreting infant emotional displays (Cardi et al., 2014a). Therefore, future studies may benefit from further exploration of the neural processes that underlie such interpretation bias.

Although, emotional infant stimuli have been found to be effective in eliciting strong emotional responses in adults, another limitation of the current study was that we did not evaluate participants' interest in or experiences with infants, which could influence responses to emotional infant stimuli. For example it has been found that people who are interested in infants display attentional bias toward infant faces over adult faces and are more motivated to view images of infants than those who are not interested in infants (Cárdenas et al., 2013; Charles et al., 2013). Future studies may benefit from assessing participants interest in and experiences with infants with self-report measures, such as the Interest in Infants Inventory (Goldberg et al., 1982; Maestripieri and Pelka, 2002).

Another limitation of the study was small sample size and lack of IQ or cognitive assessment. The small sample size prevented us from exploring further differences between AN participants who were taking SSRIs during the time of the study and those AN participants who were free of psychotropic medication. Additionally, a small number of the AN participants were receiving inpatient treatment during the study, whereas the majority of the group were volunteers and not in treatment for their ED. Therefore, the impact of SSRI medication and treatment on the group differences observed cannot be ruled out. Similarly, we did not conduct formal IQ or cognitive assessment, which could have impacted the findings. However, the AN and HC groups were matched for level of education and the task did not have a strong cognitive component. Still future studies may benefit from including larger samples and assessing the impact of the above-mentioned factors.

Finally, the AN participants in the current study were not weight recovered and therefore, it cannot be ruled out that the group differences were due to state of malnutrition. Malnutrition can, thus pose difficulties in trying to explore the neural mechanisms that underlie social–emotional processing in AN. On the other hand, including only weight recovered AN participants is not without difficulties. Different stages of illness and recovery are often associated with different challenges (Kordy et al., 2002; Treasure et al., 2015), making it difficult to generalize findings from studies including only weight recovered AN participants to people in the acute state of illness. Thus future studies may benefit from exploring the

neural processes that underlie difficulties in social–emotional functioning across different stages of illness.

CONCLUSION

The aim of the current study was to investigate differences in neural mechanisms that underlie implicit processing of emotional infant faces between AN and HC participants. The results revealed increased recruitment of the bilateral amygdala and a very small cluster in the right DLPFC in the AN group relative to the HC group while viewing positively valenced infant stimuli. Additionally, relative to the HC group, the AN participants showed increased recruitment of the left posterior insula while viewing negatively valenced infant stimuli. These findings suggest that people with AN may engage in increased prefrontal down-regulation of elevated limbic response to salient positive social–emotional stimuli, and may experience elevated subjective distress while viewing salient negative social–emotional stimuli. These neural processes may serve as useful targets for future interventions in AN, although replication of these findings with a larger sample size and an alternative task is necessary before firm conclusions can be drawn.

AUTHOR CONTRIBUTIONS

JL, YP, and KT made substantial contributions to the acquisition, analysis, or interpretation of data for the work. VC, AS, and JT made substantial contributions to the conception or design of the work. All authors were involved in drafting the work and revising it critically for important intellectual content, gave final approval of the version to be published, and are in agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpsyg.2017.00780/full#supplementary-material

FIGURE S1 | (A) Effect size (Hedges' g) map of the positively valenced > neutral infant faces contrast. Positive effect sizes indicate larger mean contrast signal change in the AN group relative to the HC group. Negative effect sizes indicate larger mean contrast signal change in the HC group relative to the AN group. **(B)** 99.9% confidence interval lower bound. **(C)** 99.9% confidence interval upper bound. Red to white colors indicate positive figures, blue to green colors indicate negative figures.

FIGURE S2 | (A) Effect size (Hedges' g) map of the negatively valenced > neutral infant faces contrast. Positive effect sizes indicate larger mean contrast signal change in the AN group relative to the HC group. Negative effect sizes indicate larger mean contrast signal change in the HC group relative to the AN group. **(B)** 99.9% confidence interval lower bound. **(C)** 99.9% confidence interval upper bound. Red to white colors indicate positive figures, blue to green colors indicate negative figures.

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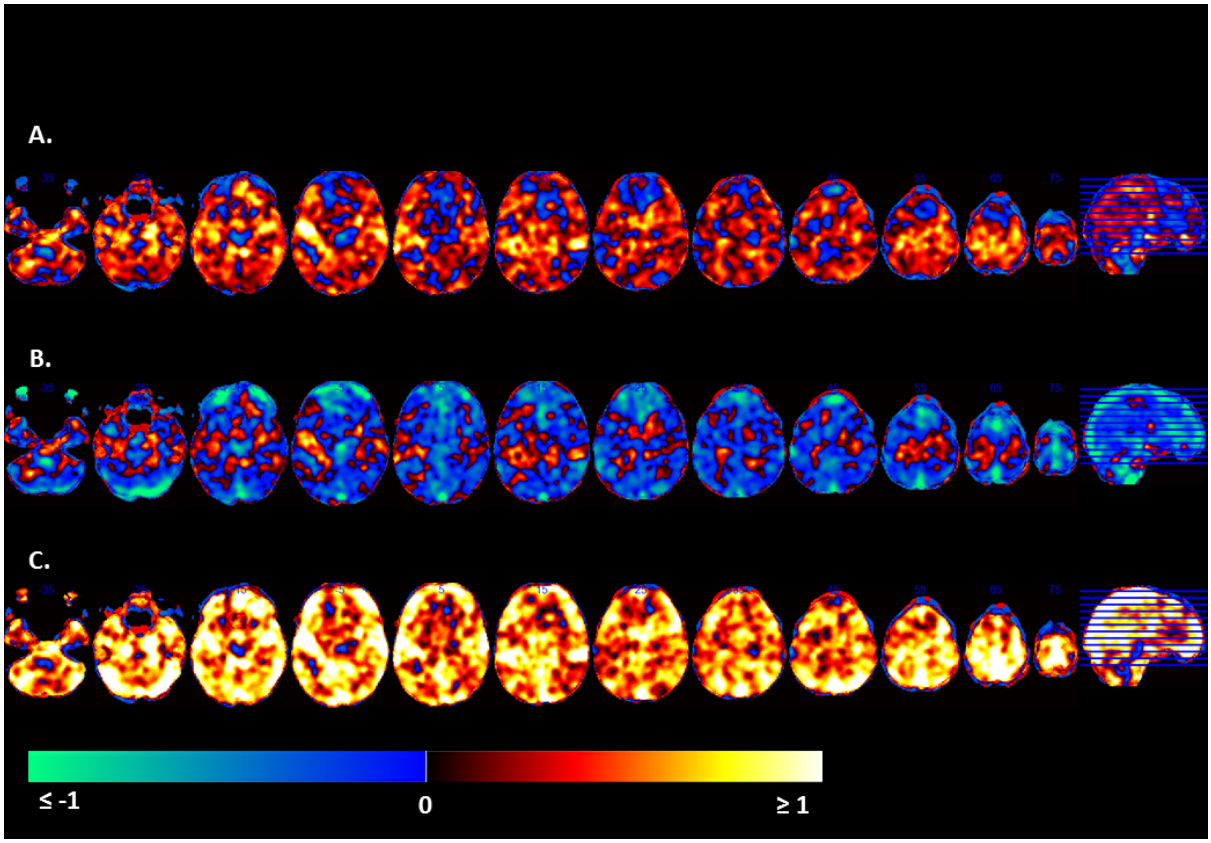
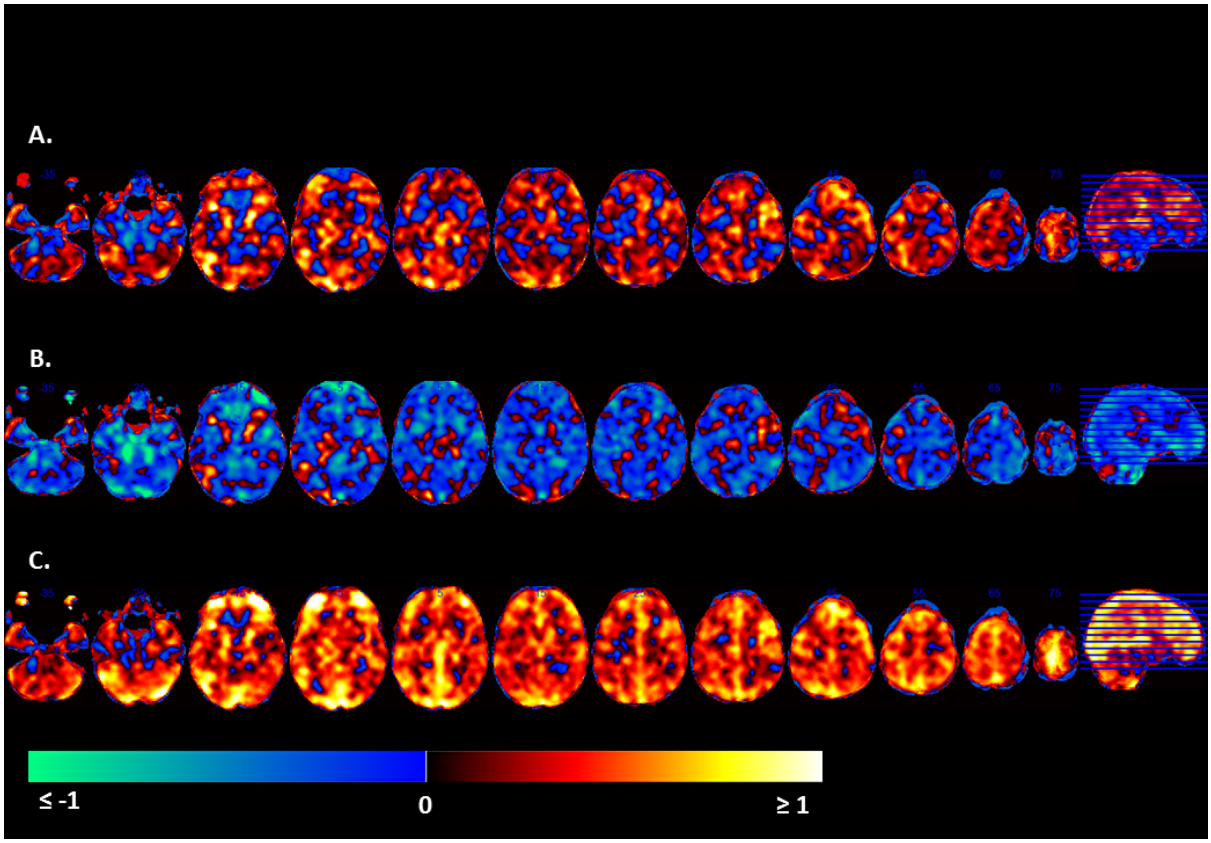
Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary Figure 1. Effect size and confidence interval maps of the positively valenced > neutral infant faces contrast.



(A) Effect size (Hedges' g) map of the positively valenced > neutral infant faces contrast. Positive effect sizes indicate larger mean contrast signal change in the AN group relative to the HC group. Negative effect sizes indicate larger mean contrast signal change in the HC group relative to the AN group. (B) 99.9% confidence interval lower bound. (C) 99.9% confidence interval upper bound. Red to white colours indicate positive figures, blue to green colours indicate negative figures.

Supplementary Figure 2. Effect size and confidence interval maps of the positively valenced > neutral infant faces contrast.



(A) Effect size (Hedges' g) map of the negatively valenced > neutral infant faces contrast. Positive effect sizes indicate larger mean contrast signal change in the AN group relative to the HC group. Negative effect sizes indicate larger mean contrast signal change in the HC group relative to the AN group. (B) 99.9% confidence interval lower bound. (C) 99.9% confidence interval upper bound. Red to white colours indicate positive figures, blue to green colours indicate negative figures.

Supplementary Table 1. Correlations between ROI mean signal change and BMI, medication status, and self-reported psychopathology in the AN group.

Contrast		ROI	BMI	Medication status	EDEQ	DASS
Positive	>	Left Amygdala	ρ = 0.31, p = 0.188	ρ = 0.07, p = 0.764	ρ = 0.30, p = 0.196	ρ <0.01, p = 0.999
Neutral		Right Amygdala	ρ = 0.13, p = 0.575	ρ = 0.15, p = 0.519	ρ = 0.43, p = 0.057	ρ = -0.14, p = 0.546
		DLPFC	ρ = -0.17, p = 0.469	ρ = 0.23, p = 0.337	ρ = -0.21, p = 0.366	ρ = -0.08, p = 0.725
Negative	>	Insula	ρ = -0.26, p = 0.259	ρ = -0.33, p = 0.151	ρ = -0.24, p = 0.307	ρ = -0.25, p = 0.292
Neutral						

ROI = region of interest; DLPFC = dorsolateral prefrontal cortex; BMI = body mass index; EDEQ = Eating Disorder Examination Questionnaire; DASS = Depression, Anxiety, and Stress Scale

CHAPTER 5:

The effects of intranasal oxytocin on smoothie intake, cortisol and attentional bias in anorexia nervosa

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The effects of intranasal oxytocin on smoothie intake, cortisol and attentional bias in anorexia nervosa

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ABSTRACT

Background: Anorexia nervosa (AN) is characterised by severe malnutrition as well as intense fear and anxiety around food and eating with associated anomalies in information processing. Previous studies have found that the neuropeptide, oxytocin, can influence eating behaviour, lower the neurobiological stress response and anxiety among clinical populations, and alter attentional processing of food and eating related images in AN.
Methodology: Thirty adult women with AN and twenty-nine healthy comparison (HC) women took part in the current study. The study used double blind, placebo controlled, crossover design to investigate the effects of a single dose of intranasal oxytocin (40 IU) on a standard laboratory smoothie challenge, and on salivary cortisol, anxiety, and attentional bias towards food images before and after the smoothie challenge in AN and HC participants. Attentional bias was assessed using a visual probe task.
Results: Relative to placebo intranasal oxytocin reduced salivary cortisol and altered anomalies in attentional bias towards food images in the AN group only. The oxytocin-induced reduction in attentional avoidance of food images correlated with oxytocin induced reduction in salivary cortisol in the AN group before the smoothie challenge. Intranasal oxytocin did not significantly alter subjective feelings of anxiety or intake during the smoothie challenge in the AN or HC groups.
Conclusions: Intranasal oxytocin may moderate the automated information processing biases in AN and reduce neurobiological stress. Further investigation of the effects of repeated administration of oxytocin on these processes as well as on eating behaviour and subjective anxiety would be of interest.

1. Introduction

Anorexia nervosa (AN) is characterised by an intense fear of food and eating with associated avoidance behaviours and severe malnourishment (American Psychiatric Association, 2013). Over time these associations become stronger and cues related to food and eating become linked to changes in brain function and information processing biases begin to develop (Schmidt and Treasure, 2006; Treasure et al., 2012; Yackobovitch-Gavan et al., 2009). To date, a number of studies have demonstrated that relative to healthy individuals, people with AN have elevated plasma and salivary cortisol levels, which suggests a dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis (Bailer and Kaye, 2003; Connan et al., 2003; Lawson et al., 2013b; Licinio et al., 1996). Furthermore, behavioural studies have found that people with AN have elevated autonomic responses to illness-related stimuli, such as food (Léonard et al., 1998; Rigaud et al., 2007; Soussignan et al., 2011; Uher et al., 2004). A study investigating the effect of a blind gastric load of 0, 300, or 700 calories on endocrine responses in AN found that the cortisol response increased with the calorie content of the gastric load in the AN participants (Rigaud et al., 2007). Further work has also demonstrated that relative to healthy participants, people with AN report greater fear and disgust in response to food stimuli, and have elevated corrugator facial EMG (i.e. frowning) and physiological arousal response to food images and when confronted

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with a test meal (Léonard et al., 1998; Soussignan et al., 2011; Uher et al., 2004).

In addition to elevated anxiety and stress around eating, people with AN also have anomalies in attention towards food (Brooks et al., 2011). A meta-analysis found that people with AN show an increased attentional bias (AB) towards food images (Brooks et al., 2011). Another study utilising eye-tracking methodology found that relative to healthy participants, people with AN showed early AB towards food images, but later avoidance of food images (Giel et al., 2011). Similarly, electroencephalogram (EEG) and magnetoencephalography (MEG) studies have found elevated early and reduced later posterior activation in people with AN compared to healthy individuals in response to food stimuli (Godier et al., 2016; Wolz et al., 2015). Interestingly, a study by Cardi et al. (2013) investigated AB towards food images before and after a standard test meal challenge and found that inpatients with AN showed greater AB towards food images following exposure to a test meal challenge. Thus, taken together, these findings suggest that anomalies in AB towards food images is a prominent feature of AN and is likely fuelled by stress and anxiety around food and eating.

Animal studies have demonstrated that the neuropeptide oxytocin is involved in many regulatory functions in the central nervous system including stress response and food intake (Cochran et al., 2013; Olszewski et al., 2010). A recent meta-analysis found that intranasal oxytocin significantly reduced cortisol response to stressful stimuli among clinical populations of people suffering from a range of disorders, including depression, substance dependence, fragile X syndrome and borderline personality disorder, characterised by a chronic dysregulated and hyperactivated HPA axis (Cardoso et al., 2014). Additionally, emerging evidence from animal studies has demonstrated that central administration of an oxytocin receptor agonist can normalise novelty-induced decrease in food intake in mice, suggesting that oxytocin may play a role in moderating the influence of stress and anxiety on eating (Olszewski et al., 2014). Thus intranasal oxytocin may be helpful facilitating exposure treatment for food restriction fuelled by anxiety.

There has been increasing interest in the possibility that oxytocin may be involved in the pathophysiology of eating disorders (Maguire et al., 2013). A recent meta-analysis reported that peripheral and cerebrospinal fluid (CSF) levels of endogenous oxytocin were significantly lower in AN relative to normal weight controls, which the authors suggested is likely associated with dysregulation of the HPA axis (Rutigliano et al., 2016). Additionally, another study has reported atypical, elevated oxytocin response to food intake in AN, which was associated with pre-meal hypoactivation of the hypothalamus, orbitofrontal cortex, insula, and amygdala in response to food images in AN relative to healthy participants (Lawson et al., 2013b; Lawson et al., 2012). Moreover, a recent proof of concept study found that a single dose of 40IU of intranasal oxytocin reduced anomalies in AB towards images of food, eating, and negative body shape in people with AN (Kim et al., 2014a). The authors also found that intranasal oxytocin led to a reduction in caloric intake in the 24 h following administration in participants with bulimia nervosa (Kim et al., 2015). These findings suggest that intranasal oxytocin can alter anomalies in attentional processes to specific and general aversive stimuli in AN and may alter eating behaviour.

The aim of the current study was to examine the impact of a single dose of intranasal oxytocin on a standard smoothie challenge, as well as neurobiological stress and AB towards food images, as measured with a visual probe task, before and after the smoothie challenge in adult women with AN and gender and age matched healthy control (HC) participants. Based on previous findings outlined above, we hypothesised that relative to HCs, participants with AN would consume less during the smoothie challenge, have elevated anxiety and salivary cortisol, and anomalies in AB towards food images before and after the smoothie challenge. Additionally, we hypothesised that oxytocin administration would decrease anxiety, increase smoothie intake, reduce cortisol and alter anomalies in AB towards food images in participants with AN.

2. Methods and materials

2.1. Participants

Fifty-nine women participated in the study. Thirty women met the DSM-5 criteria for AN, with mean BMI of 16.30 (SD = 2.04) and age of 26.20 (SD = 6.82). Diagnosis was confirmed using the Structured Clinical Interview for DSM-5 (First et al., 2015). Fifteen of the AN participants were recruited from the South London and Maudsley NHS Foundation Trust inpatient unit and were in treatment. The other fifteen AN participants were recruited through ED charities (BEAT, Succeed). Fifteen of the AN participants were taking medication (anti-depressants) during the study.

The HC participants (N = 29), with mean BMI of 23.25 (SD = 3.65) and age of 26.83 (SD = 8.54), were recruited from the community and amongst King's College London students and staff. HC participants were of normal weight and were screened for current or past psychiatric disorders, and alcohol or drug misuse with the Structured Clinical Interview for DSM-5 (First et al., 2015). Participants were excluded from the study if they reported medical or psychiatric problems, a history of or current alcohol or drug abuse, current impairments in cardiovascular functioning, pregnancy or plans to become pregnant during the study. Prior to participating in the study, all participants gave a written informed consent. Ethical approval for the study was obtained from National Research Ethics Service (NRES) committee (14/LO/0128) and all procedures were conducted in accordance with the latest declaration of Helsinki (2008).

The sample size was based on power analysis conducted with G*Power for a repeated measures design (Faul et al., 2007). To ensure adequate power (0.80) to detect an effect in the mixed model, the total recommended sample size was 60 participants.

2.2. Experimental design

The study employed a double blind, placebo controlled, within subjects, crossover design. All participants received a single dose of both oxytocin and placebo in separate sessions. The treatment order was pseudo-randomised so that half of the AN participants and half of the HC participants received oxytocin in the first session and the other half of the AN and HC participants received oxytocin in the second session. Only the Maudsley pharmacy, responsible for dispensing the compounds were aware of the order in which each participant received the compounds. The experimenter and the participants were both blind to treatment order.

The study flow chart is presented in Supplementary Fig. S1 . Prior to administration of the compounds participants were asked to provide a saliva sample for cortisol analysis, and to provide a baseline rating of anxiety on a visual analogue scale (VAS) (for further details see Supplementary information). The intranasal oxytocin and the placebo were self-administered by the participants in ten sprays, five sprays in each nostril every 45 s (for further details see Supplementary information). Fifty minutes after administration, participants gave the second saliva sample and VAS anxiety rating. The first visual probe task was then administered approximately 55 min after administration of the compound (T1; for further details see Supplementary information). This was followed by the smoothie challenge consisting of a 250 ml fruit smoothie (a choice from 3 Innocent smoothie flavours: strawberries and bananas, mangoes and passion fruits, and kiwis, apples and limes).

All labels were kept on the bottles and this contained the calorie content and nutritional information. The participants were encouraged to consume as much of the smoothie as they liked under non-supervised conditions and were left for 15 min to do this. If participants refused to have a smoothie they were informed that they did not have to have it, but were encouraged to try it and left with the smoothie for the duration of the challenge. Following this, participants gave a third VAS anxiety rating and the second visual probe task was administered, approximately 75 min after administration of the compound (T2; for further details see Supplementary information). Finally, the third saliva sample was collected approximately 80 min after administration. The timing was based on previous work investigating the effects of intranasal oxytocin on AB in eating disorders and on cerebral blood flow among healthy individuals (Kim et al., 2014a; Paloyelis et al., 2016).

The two sessions were scheduled a minimum of one day and maximum of five days apart. All HC and AN participants who were menstruating regularly during the study were tested during the follicular phase, 1–15 days following the onset of a menstrual cycle. All participants were asked to refrain from consuming any alcoholic or caffeinated beverages twelve hours prior to the sessions. Additionally, the participants were asked to not consume any food two hours prior to the sessions, in order to minimise effects of fullness and hunger on the tasks.

2.3. Visual probe task

A visual probe detection task was used to investigate AB towards and away from food and neutral, non-food images in AN. The task was amended from that used in a previous study (Cardi et al., 2013), and the food images were acquired from a standardised set of images and depicted a variety of savoury and sweet food items on a blue background (Uher et al., 2004). The food images depicted highly palatable food, and were matched for size and calorie content. The non-food images consisted of neutral images of furniture. In the visual probe detection task participants were presented with two images simultaneously on a computer screen side by side for 500 ms. One of the images then revealed one of two visual probes underneath them and participants were instructed to press specific keys on the keyboard to indicate which probe they saw. The probe was presented until participants responded. The task was presented with E-prime software (Psychology Software Tools, Sharpsburg, USA) and consisted of 96 trials. The task lasted approximately 3 min. Further details regarding the visual probe task are presented in Supplementary information.

2.4. Statistical analysis

All data was analysed using Stata 14 (StataCorp, 2015) and $p < 0.05$ was considered significant. The self-report questionnaire data were analysed with non-parametric two-sample median Chi^2 tests. Effect sizes were estimated with Cramer's ϕ, which can be interpreted as small (0.10), medium (0.30), or large (0.50) (Cohen, 1988).

Due to the highly skewed nature of the smoothie data, logarithm 10 transformation was performed prior to analysis. Group differences in smoothie intake in millilitres (ml) consumed as well as the effect of oxytocin/placebo on consumption were then analysed with a bootstrapped mixed linear model with 1000 repetitions using the *bootstrap* and *mixed* functions in Stata (StataCorp, 2015). Significant interactions were further investigated by computing post-hoc contrasts and pairwise comparisons.

The AB data was analysed separately for the 500 ms and 1250 ms ITI blocks with a $2 \times 2 \times 2$ mixed linear model using the *mixed* function with bootstrapping (1000 repetitions) in Stata 14 (StataCorp, 2015). Drug (oxytocin, placebo), time (before smoothie challenge, after smoothie challenge), and group (AN, HC) were entered as fixed effects with a random intercept and random slope on drug. Similarly, the VAS anxiety and salivary cortisol data were analysed with a $2 \times 3 \times 2$ bootstrapped mixed model with drug (oxytocin, placebo), time (baseline, before smoothie challenge, after smoothie challenge), and group (AN, HC) and a random intercept and random slope on drug. Significant interactions were explored further by calculating contrasts and pairwise post-hoc comparisons using the *contrast* and *pwcompare* functions (StataCorp, 2015).

We additionally explored whether oxytocin induced changes in AB scores correlated with oxytocin induced changes in VAS anxiety ratings and salivary cortisol before and after the smoothie challenge within the AN group. AB delta scores were calculated by first adding a constant to all AB data to ensure no AB scores were negative. The AB scores from the placebo session were then subtracted from the AB scores from the oxytocin session. Thus, negative scores indicated greater AB towards food images in the placebo session and positive scores indicated greater AB towards food images in the oxytocin session. VAS anxiety and salivary cortisol delta scores were calculated in a similar manner. We then conducted correlation analyses to investigate significant correlations between the deltas in Stata using the *spearman* function (StataCorp, 2015).

Effects of medication status on AB, salivary cortisol, and VAS anxiety ratings as well as on the effects of oxytocin are presented in Supplementary information.

3. Results

3.1. Demographic and clinical characteristics

The clinical and self-report questionnaire data is presented in Table 1 and was corrected for multiple comparisons with the false discovery rate set at $q < 0.05$ (Benjamini and Hochberg, 1995). Following correction, $p < 0.04$ was considered significant. The AN and HC groups were matched for age, and as expected the AN group had lower BMI than the HC group. The AN group also scored higher on the EDEQ reporting higher levels of restraint, eating concern, weight concern, and shape concern compared to the HC group. Additionally, the AN group scored higher on the DASS reporting elevated levels of depression, anxiety, and stress relative to the HC group.

3.2. Smoothie intake

Smoothie intake in the oxytocin and placebo conditions are summarised separately for each group in Table 2 (see Supplementary Fig. S2). The mixed model revealed a significant effect of Group, with HC participants consuming significantly more smoothie during the challenge than AN participants across conditions. There was no significant effect of Drug or Drug x Group interaction. Eleven AN participants did not consume any of the smoothie in the placebo condition, and nine of these eleven AN participants also did not consume any smoothie in the oxytocin condition.

3.3. AB: 500 ms ITI blocks

The AB scores in the 500 ms ITI blocks among the AN and HC groups following oxytocin and placebo administration are presented in Table 3 (see Supplementary Fig. S3A,B). P-values were corrected for multiple comparisons with the false discovery rate set at $q < 0.05$ (Benjamini and Hochberg, 1995). Following correction, $p < 0.02$ was considered significant.

The mixed model revealed a significant Drug x Time x Group interactions (Table 3). The 3-way interaction was further investigated in three ways. First we explored the Drug x Time interaction separately within the AN and HC groups. The results revealed

Table 1
Clinical and demographic sample characteristics.

	AN (N = 30) Median	Q1	Q3	HC (N = 29) Median	Q1	Q3	AN vs HC X^2 statistic, p value	Cramer's ϕ
BMI	16.13	14.56	18.14	22.21	20.78	25.51	$X^2 = 35.55$, $p < 0.001$	0.78
Age	24.50	22.00	28.25	25.00	23.00	27.50	$X^2 = 0.02$, $p = 0.875$	0.02
EDEQ total	4.13	3.00	5.13	0.59	0.34	1.00	$X^2 = 35.27$, $p < 0.001$	0.77
EDEQ restraint	3.70	2.60	5.05	0.60	0.00	1.70	$X^2 = 20.79$, $p < 0.001$	0.59
EDEQ weight concern	4.20	2.70	5.65	0.60	0.30	1.40	$X^2 = 31.38$, $p < 0.001$	0.73
EDEQ shape concern	4.86	3.56	5.91	0.75	0.38	1.38	$X^2 = 41.67$, $p < 0.001$	0.84
EDEQ eating concern	4.10	2.90	4.80	0.20	0.00	0.40	$X^2 = 41.67$, $p < 0.001$	0.84
DASS Total	63.00	40.00	86.00	4.00	1.00	10.00	$X^2 = 37.49$, $p < 0.001$	0.80
DASS Depression	21.00	13.50	32.00	0.00	0.00	2.00	$X^2 = 26.61$, $p < 0.001$	0.67
DASS Anxiety	12.00	6.00	21.00	0.00	0.00	2.00	$X^2 = 29.07$, $p < 0.001$	0.70
DASS Stress	25.00	17.50	31.00	2.00	0.00	7.00	$X^2 = 37.49$, $p < 0.001$	0.80

AN = anorexia nervosa, HC = healthy comparison, BMI = Body mass index, EDEQ = Eating Disorder Examination Questionnaire, DASS = Depression, Anxiety, and Stress Scale.

Table 2
Smoothie intake in millilitres (ml) in the AN and HC groups in the oxytocin and placebo conditions.

Drug	AN (N = 30) Mean (SD)	HC (N = 29) Mean (SD)	X^2 statistic, p value
Oxytocin	113.10 (102.94)	224.24 (47.58)	Drug: $X^2 = 1.39$, $p = 0.239$
Placebo	99.07 (102.16)	237.52 (30.29)	Group: $X^2 = 131.66$, $p < 0.001$
			Drug x Group: $X^2 < 0.01$, $p = 0.975$

AN = anorexia nervosa, HC = healthy comparison.

Table 3
AB towards food images before and after the test meal in the AN and HC groups.

ITI	Time	Drug	AN (N = 30) Mean (SD)	HC (N = 29) Mean (SD)	X^2 statistic, p value
500 ms	Before test meal	Oxytocin	−2.66 (33.57)	2.52 (36.79)	Drug: $X^2 = 1.41$, $p = 0.234$
		Placebo	−13.69 (29.27)	13.86 (42.70)	Time: $X^2 = 0.08$, $p = 0.780$
	After test meal	Oxytocin	−13.64 (38.78)	0.41 (43.66)	Group: $X^2 = 3.47$, $p = 0.063$
		Placebo	10.64 (34.42)	−2.99 (42.55)	Drug x Time: $X^2 = 0.09$, $p = 0.764$
					Drug x Group: $X^2 = 1.06$, $p = 0.302$
					Time x Group: $X^2 = 2.81$, $p = 0.093$
					Drug x Time x Group: $X^2 = 6.49$, $p = 0.011$
1250 ms	Before test meal	Oxytocin	−5.06 (30.85)	8.66 (43.47)	Drug: $X^2 = 0.41$, $p = 0.524$
		Placebo	2.64 (34.08)	−4.53 (35.44)	Time: $X^2 = 1.97$, $p = 0.160$
	After test meal	Oxytocin	−6.78 (48.86)	−5.24 (67.38)	Group: $X^2 = 0.01$, $p = 0.925$
		Placebo	−8.13 (41.65)	−13.95 (44.29)	Drug x Time: $X^2 = 0.03$, $p = 0.863$
					Drug x Group: $X^2 = 1.47$, $p = 0.226$
					Time x Group: $X^2 = 0.18$, $p = 0.669$
					Drug x Time x Group: $X^2 = 0.29$, $p = 0.591$

AN = anorexia nervosa, HC = healthy comparison, AB = Attentional bias, ITI = inter-trial interval.

that the Drug x Time interaction was significant within the AN group ($X^2 = 7.78$, $p = 0.005$), but not within the HC group ($X^2 = 0.94$, $p = 0.333$). The interaction was then further investigated within the AN group by exploring the effects of Drug at T1 and T2. The results revealed that oxytocin administration significantly reduced AB towards food images in the AN group at T2, after the smoothie challenge, ($Z = -2.72$, $p = 0.007$, 95% CI [−41.78, −6.78]), but not at T1, before the smoothie challenge al, ($Z = 1.38$, $p = 0.168$, 95% CI [−4.66, 26.72]).

Finally, we explored the Group x Time interaction separately in the oxytocin and placebo conditions. The results revealed a significant Group x Time interaction only in the placebo condition ($X^2 = 10.03$, $p = 0.002$), but not in the oxytocin condition ($X^2 = 0.38$, $p = 0.540$). When group differences were explored further, the results revealed that AN participants showed significantly more AB away from food images than HC participants at T1, before the smoothie challenge, ($Z = -3.08$, $p = 0.002$, 95% CI [−45.09, −10.02]), but not at T2, after the smoothie challenge, ($Z = 1.56$, $p = 0.118$, 95% CI [−3.46, 30.71]).

We then explored the Group x Drug interaction separately at T1 and T2, which revealed no significant interaction at T1, before the smoothie challenge ($X^2 = 3.06$, $p = 0.080$), or at T2, after the smoothie challenge ($X^2 = 4.13$, $p = 0.042$).

3.4. AB: 1250 ms ITI blocks

The attentional bias scores in the 1250 ms ITI blocks among the AN and HC groups following oxytocin and placebo administration are presented in Supplementary Fig. S4A,B. The drug x time x group mixed model revealed no significant effects or interactions in the 1250 ms ITI blocks (Supplementary Table S1).

3.5. Salivary cortisol

Mean salivary cortisol levels are presented separately for each group in the oxytocin and placebo conditions in Table 4 (see Supplementary Fig. S5). The mixed model revealed a significant effect of Group, with AN participants having significantly higher cortisol levels than HC participants across conditions and time points (Table 4).

The mixed model additionally revealed a significant Drug x Group interaction. The interaction was further explored by exploring the effect of Drug on salivary cortisol separately in each group. The results revealed a significant effect of Drug in the AN group ($Z = -1.99$, $p = 0.046$, 95% CI [−1.30, −0.01]), with AN participants having lower salivary cortisol levels in the oxytocin condition. There was no significant difference between oxytocin

Table 4
Salivary cortisol at each time point in the AN and HC groups.

Time	Drug	AN (N = 30) Mean (SD)	HC (N = 29) Mean (SD)	X^2 statistic, p value
Baseline	Oxytocin	5.35 (5.66)	3.71 (3.68)	Drug: $X^2 = 0.37$, p = 0.542
	Placebo	5.35 (3.31)	3.18 (2.67)	Time: $X^2 = 6.91$, p = 0.032
Before smoothie challenge	Oxytocin	4.81 (3.35)	2.57 (2.10)	Group: $X^2 = 179.21$, p < 0.001
	Placebo	5.54 (4.04)	2.24 (1.75)	Drug x Time: $X^2 = 1.46$, p = 0.482
After smoothie challenge	Oxytocin	4.69 (3.14)	2.59 (1.92)	Drug x Group: $X^2 = 7.22$, p = 0.007
	Placebo	5.93 (4.48)	2.09 (1.57)	Time x Group: $X^2 = 4.33$, p = 0.115
				Drug x Time x Group: $X^2 = 1.38$, p = 0.502

AN = anorexia nervosa, HC = healthy comparison.

and placebo in the HC group (Z = 1.94, p = 0.052, 95% CI [−0.004, 0.84]).

3.6. Self-reported VAS anxiety ratings

Mean VAS anxiety ratings are presented separately for each group in the oxytocin and placebo conditions in Supplementary Fig. S6 . The mixed model exploring group differences in anxiety ratings at different time points in the oxytocin and placebo conditions revealed a significant effect of Time (Supplementary Table S2). VAS anxiety ratings were significantly higher after the smoothie challenge than before the smoothie challenge across groups (Z = 3.62, p < 0.001, 95% CI [0.03, 0.09]). There were no significant differences in self-reported anxiety at baseline and before the smoothie challenge (Z = −1.85, p = 0.064, 95% CI [−0.07, 0.002]) or at baseline and after the smoothie challenge (Z = 1.21, p = 0.224, 95% CI [−0.01, 0.06]).

3.7. Correlations between oxytocin-induced changes in AB, anxiety, and salivary cortisol

The correlational analysis revealed a significant inverse correlation between salivary cortisol delta scores and oxytocin-induced changes in AB before the smoothie challenge (ρ = −0.41, p = 0.026). Inverse correlation between VAS anxiety delta scores and oxytocin induced changes in AB before the smoothie challenge also approached significance (ρ = −0.31, p = 0.097). There were no significant correlations between oxytocin-induced changes in AB and cortisol delta scores (ρ = −0.11, p = 0.574), anxiety delta scores (ρ = −0.08, p = 0.692) after the smoothie challenge, or BMI (before smoothie challenge: ρ = 0.09, p = 0.631, after smoothie challenge: ρ = 0.06, p = 0.752).

4. Discussion

The aim of the current study was to examine the effects of a single dose of intranasal oxytocin on smoothie intake during a standard smoothie challenge as well as on salivary cortisol and AB towards food images before and after the smoothie challenge. As hypothesised, relative to HCs, the majority of the AN participants consumed less smoothie during the smoothie challenge and had elevated anxiety and salivary cortisol levels, and showed anomalies in AB towards food images. Contrary to what was hypothesised, oxytocin administration did not significantly influence smoothie intake among the AN or HC participants nor did it moderate subjective anxiety. However as postulated, oxytocin administration significantly reduced salivary cortisol and reduced the anomalies in AB towards food images in people with AN. Additionally, correlational analysis revealed an association between oxytocin-induced reduction in salivary cortisol and attentional avoidance of food images before the smoothie challenge.

The current findings revealed that relative to the HC group, the AN participants had significantly higher salivary cortisol, and intranasal oxytocin reduced salivary cortisol levels across time points in the AN group only. This supports findings from a recent 6-week pilot trial that reported reductions in salivary cortisol levels and anticipatory cortisol response to an afternoon snack among inpatients with AN following repeated twice-daily administration of 18IU of intranasal oxytocin (Russell et al., 2013). This is also consistent with findings from a recent meta-analysis, which found that intranasal oxytocin reduced the cortisol response to stressful stimuli among clinical populations, with conditions characterised by dysregulation of the HPA axis (Cardoso et al., 2014). These findings suggest that oxytocin is able to moderate both acute and chronic stress response among clinical populations. Previous work has suggested that increased availability of another hypothalamic neuropeptide, vasopressin, plays an important role in dysregulated and elevated neurobiological stress response in AN (Connan et al., 2003). Oxytocin may moderate this system by reducing vasopressin availability, thus, reducing elevated stress response (Heinrichs and Domes, 2008; Heinrichs et al., 2009). Another possibility is that the dysregulation of the HPA axis and associated elevated stress response are due to a failure in the stress-induced up-regulation of endogenous oxytocin (Zheng et al., 2010). Intranasal oxytocin may then moderate the stress response by regulating HPA axis activity (Neumann and Slattery, 2016). Although the exact mechanism of the effects of oxytocin on neurobiological stress is still not clear and need further exploration, these findings raise the possibility that oxytocin might have a role as a neuroenhancer that facilitates extinction learning as has been proposed based on recent findings from animal studies (Eckstein et al., 2015; Hofmann et al., 2015; Stockhorst and Antov, 2016).

The current study also found that relative to HCs, AN participants had anomalies in AB towards food images, showing greater avoidance of food images before the smoothie challenge and greater bias towards food images after the smoothie challenge, although this failed to reach significance. Oxytocin administration altered these anomalies significantly reducing bias towards food images after the smoothie challenge in the AN group. The moderation of the anomalies in AB toward food are in keeping with previous work exploring the effects of intranasal oxytocin on attentional processing in AN (Kim et al., 2014a; Kim et al., 2014b). The authors reported that intranasal oxytocin significantly reduced bias towards illness-related stimuli, including images of food, eating, fat body shape, and disgusted faces in AN (Kim et al., 2014a; Kim et al., 2014b). These findings suggest that intranasal oxytocin can alter automated attentional processing of illness-related stimuli, which would otherwise be difficult to target through talking therapies. Additionally, it has recently been suggested that oxytocin may be a useful supplement to treatment as usual in eating disorders (Maguire et al., 2013; Treasure et al., 2015).

The current study also attempted to explore the mechanism of the effects of oxytocin on attentional processes. The findings revealed a significant inverse correlation between oxytocin-induced reduction in AB away from food images and oxytocin-induced reduction in salivary cortisol before the smoothie challenge. These findings suggest that as intranasal oxytocin reduced salivary cortisol, it also reduced attentional avoidance

of food images before the smoothie challenge. This is in accordance with findings from animal studies demonstrating that central oxytocin release reduces neurobiological stress and stress-related behaviours, such as hiding during an open field test and periods of immobility during forced swimming test (Chaviaras et al., 2010; Zheng et al., 2010). These findings are also supported by a previous functional neuroimaging study, which found that intranasal oxytocin normalises anxiety-induced amygdala hyper-reactivity to threatening, illness related stimuli in generalised social anxiety disorder (Labuschagne et al., 2010). Furthermore, among people with fragile X syndrome, characterised by autism-like difficulties in social processing, intranasal oxytocin has been shown to reduce salivary cortisol and improve eye gaze during social interaction (Hall et al., 2012). However, it is of note that in the current study we were not able to find a significant correlation between oxytocin-induced changes in AB towards food images and salivary cortisol or self-reported anxiety, which may have been due to timing of the final saliva sample collected after the smoothie challenge (Hellhammer et al., 2009). Therefore, further research using functional neuroimaging techniques are needed to further examine the mechanism through which intranasal oxytocin alters attentional processes in AN.

Oxytocin administration did not significantly alter smoothie intake in either the AN or HC group in the current study. Previous findings from studies investigating the effects of a single dose of intranasal oxytocin on eating behaviour in humans are variable (Olszewski et al., 2016). One study reported that intranasal oxytocin reduced food intake during a buffet test meal in healthy men following an overnight fast (Lawson et al., 2015). On the other hand, another study failed to find significant oxytocin-induced reduction in food intake during a buffet breakfast following an overnight fast (Ott et al., 2013). Instead the authors reported that intranasal oxytocin reduced hedonic snacking two hours after the test meal (Ott et al., 2013). Interestingly, the buffet test meal in the Lawson et al. (2015) had more variety and contained more options than that in the Ott et al. (2013) study. Variety has been shown to be an important factor increasing hedonic value of food and intake during buffet test meals (Brondel et al., 2009; Hetherington et al., 2006). The current study conducted in the afternoon and participants had not consumed any food for at least three hours by the time they were introduced to the test meal consisting of a single fruit smoothie. It is probable that clinical status, hedonic hunger, and the context of the test meal may influence the effect of oxytocin on eating.

Considering the important role that chronic neurobiological stress and information processing biases play in the maintenance of disordered eating, it was perhaps surprising that oxytocin-induced reduction in salivary cortisol and in automated AB towards food images did not translate into greater intake during the smoothie challenge and less anxiety throughout the session. It is possible that a test meal disguised as a "taste test" might be a more sensitive outcome as it would allow a covert assessment of intake of various different types of food and micronutrients. Additionally, using ecologically valid assessments such as 24-h food diaries could help to further investigate the effects of intranasal oxytocin on food intake under naturalistic settings. Indeed, a recent proof of concept study by Kim et al. (2015), which used 24-h food diaries, found that a single dose of intranasal oxytocin reduced caloric intake among people with bulimia nervosa. The authors did not find any increase in caloric intake among people with AN (Kim et al., 2015). However, it is of not that the AN participants in the Kim et al. (2015) study were all inpatients undergoing treatment. Thus, further investigation of the effects of intranasal oxytocin on food intake among people with AN under naturalistic conditions would be necessary before recommendations regarding its potential as an add-on treatment can be made.

Finally, the demographic data, including questionnaire responses, age, and BMI, in the current study was skewed. However this is not unusual with questionnaire and other measures that contain natural boundaries, below or above which the data can't reach (Diez et al., 2015). For instance, the age data is skewed because in the current study we only recruited adults over the age of 18. Therefore, we have a natural boundary at 18 years and for this reason the data has a right skew. Similarly, a person's BMI cannot be below a certain point, but there is no clear upper limit, meaning that the data has a natural right skew.

4.1. Limitations

One of the limitations of the current study was the smoothie challenge used in the current study, which differed from test meal challenges employed in other studies (Khalsa et al., 2015; Lawson et al., 2013a; Steinglass et al., 2014). The fruit smoothies are marketed as healthy and previous studies found them to be acceptable for the AN participants (Cardi et al., 2013). However, the smoothie challenge consisted of a single 250 ml smoothie, which in some cases led to ceiling effects. Future studies may benefit from employing test meals that include both high and low calorie options. Additionally, since the smoothie options varied slightly in their calorie content (range: 126–140 Kcal), we were not able to validly analyse the data in calories consumed during the challenge. Future studies using similar smoothie challenge paradigm may benefit from matching the smoothies offered for caloric content.

Additionally, the type of visual probe task used to measure attention in the current study precluded the measurement of timing related changes in AB. Future research may benefit from exploring the effects of oxytocin on early and late attentional processes in AN using eye tracking and functional neuroimaging techniques such as EEG and MEG. Furthermore, the neutral images consisted of pictures of furniture and rooms, which were not similarly standardised as the food images. Although, prior to the study, effort was taken to ensure the images were as visually appealing as the food images, in terms of colour and contrast, this may have added noise to the data. It has also been recently proposed that standardised images should be used in order to improve comparability of studies and increase replicability and reproducibility of findings (Blechert et al., 2014).

Further, the sessions were very close together. Although, this was unlikely to influence the results of the rapid behavioural task and biological measures, it would be beneficial for the current findings to be replicated with a larger study using a longer period between the sessions.

Finally, salivary cortisol measures were taken only at three different time points. It is possible that we did not find a correlation between oxytocin-induced changes in salivary cortisol and AB after the smoothie challenge because the saliva sample was taken too soon after the challenge. It has been suggested that relative to psychological responses, there can be considerable lag in the cortisol response to stressful, anxiety-provoking stimuli (Hellhammer et al., 2009). Future research would benefits from building on these findings and further investigating the timeline of the effects of oxytocin on stress in AN and how these effects may correlate with psychological responses.

5. Conclusions

The current study examined the effects of a single dose of intranasal oxytocin on a smoothie challenge, salivary cortisol, and AB towards food images in AN. The findings revealed that intranasal oxytocin did not significantly increase smoothie intake during the smoothie challenge, but did significantly reduce salivary cortisol in

the AN group. Oxytocin did influence food-related attentional bias, which correlated with oxytocin-induced reduction in salivary cortisol in the AN group. Taken together, these findings suggest that the oxytocin may be helpful in altering automatic information processing biases and inhibiting the hyperactivation of the HPA axis and may enhance the therapeutic benefits of food exposure paradigms in anorexia nervosa. Still, it will be necessary to further examine the exact mechanism through which oxytocin exerts its effects in AN as well as the effects of chronic administration on illness related processes and eating behaviours over the long term, before any recommendation can be made regarding whether intranasal oxytocin could be introduced into treatment of AN.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2017.01.017.

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Supplementary materials.

Methodology

Compounds

The oxytocin compound consisted of 40IU of synthetic oxytocin (Syntocinon; Novartis Pharmaceutical, Basel, Switzerland). The placebo compound was designed to be identical to the oxytocin compound minus the active ingredient. Both compounds were stored in 5mL nasal spray bottles, and before use oxytocin and placebo sprays were stored in a medicine fridge (4°C) in the Maudsley Pharmacy located in Maudsley Hospital.

Self-report and clinical measures

The 36-item Eating Disorders Examination Questionnaire (EDEQ) was used to assess eating disorder psychopathology (Fairburn and Beglin, 1994). In addition to total score, the EDEQ provides measures of restraint, eating concern, weight concern, and shape concern. In the current study, the internal consistency of the EDEQ was high (Cronbach's $\alpha = 0.98$).

The 21-item Depression, Anxiety, and Stress Scale (DASS) was used to assess self-reported depression, anxiety, and stress (Lovibond and Lovibond, 1995). In the current study, the internal consistency of the DASS was high (Cronbach's $\alpha = 0.98$).

A visual analogue scale (VAS; appendix) was administered to assess self-reported anxiety levels at three different time points in both sessions (Figure 1). The internal consistency of the VAS anxiety measure was high (Cronbach's $\alpha = 0.95$).

Visual probe task

Trials in the visual probe task were presented in two alternating blocks to further investigate the nature of AB in AN. In block one the inter-trial interval (ITI) was 500ms with the trials following each other in a rapid progression. When trials are presented in rapid progression participants' performance is likely to be affected by proactive interference from the proceeding trials (Cermak, 1970; Shipstead and Engle, 2013). When more time is allowed to elapse between the trials the previous trial's interference on participants' performance on the subsequent trial is minimised (Cermak, 1970; Shipstead and Engle, 2013). Proactive interference negatively influences participants' ability to hold information regarding the previous trial in working memory (Shipstead and Engle, 2013). Therefore, the block in which the trials were presented in rapid progression allowed us to investigate more automated responding in any given trial without influences from the memory of the previous trial. Additionally, because the two ITI blocks allowed us to measure different kinds of responses to the food and non-food images, the data were analysed separately.

The visual probe task consisted of 96 trials: 48 trials with 500ms ITI and 48 trials with 1250ms ITI. The probe was two dots presented either horizontally or vertically. The participants were asked to press the "Z" key on the keyboard when they saw the

dots presented horizontally and the "Q" key when they saw the dots presented vertically.

Attentional bias scores were calculated by subtracting the reaction times in trials where a probe was revealed underneath a neutral image from reaction times in trials where a probe was revealed underneath a food image. Positive scores indicate attentional bias towards food images and negative scores indicate AB away from food images.

Salivary cortisol:

The saliva samples were collected using Salivette sampling device (Sarstedt Ltd, Leicester, England). Saliva samples instead of plasma samples were used due to its relatively non-invasive nature. Additionally, saliva cortisol and plasma cortisol levels demonstrate high agreement, and because free cortisol enters saliva through passive diffusion saliva cortisol levels are unaffected by salivary flow rate (Kirschbaum, 2000).

The samples were frozen at -20°C within 4 hours of collection until analysed. The analysis was conducted using the Salimetrics salivary cortisol enzyme competitive immunoassay kit (ELISA, Salimetrics, Inc. PA, United States, www.salimetrics.com). The microtiter plates were incubated for 1 hour at room temperature during the first incubation. When the substrate was added, the plates were incubated for half an hour in the dark at the same temperature as in the previous incubation. The amount

of cortisol enzyme conjugation was assessed using a standard plate optical density reader set at 450 nm.

Statistical analysis:

Differences between medicated and non-medicated AN participants in clinical characteristic were investigated with median Chi-squared tests (StataCorp, 2015). Effect sizes were estimated with Cramer's ϕ, which can be interpreted as small (0.10), medium (0.30), or large (0.50) (Cohen, 1988).

Due to the severely skewed nature of the VAS anxiety data, the data was transformed with logarithm 10 transformation prior to analysis. To explore potential confounding effects of anti-depressant medication on AB, salivary cortisol, or VAS anxiety ratings, we conducted another bootstrapped mixed model within the AN group using the *mixed* command (StataCorp, 2015). Furthermore, to control for differences between medicated and non-medicated AN participants arising from psychopathology, a composite psychopathology score was created by summing the DASS and EDEQ total scores. Medication status (medicated, non-medicated), drug (oxytocin, placebo), and time (T1, T2/baseline, before smoothie, after smoothie) were entered as fixed effects with a random intercept and random slope on drug and controlling for self-reported psychopathology. Significant interactions were explored further by calculating contrasts and pairwise post-hoc comparisons using the *contrast* and *pwcompare* functions (StataCorp, 2015).

Results

Clinical sample characteristics:

The clinical sample characteristics are presented in Supplementary table 3 and were corrected for multiple comparisons with the false discovery rate set at $q < 0.05$ (Benjamini and Hochberg, 1995). Following correction $p < 0.03$ was considered significant. There was a significant difference between the medicated and non-medicated AN participants in eating disorder psychopathology reporting significantly more eating concern, weight concern, and shape concern, than the non-medicated AN participants. The medicated AN participants also reported significantly more depression than the non-medicated AN participants.

Effect of anti-depressant medication on AB:

The AB scores among the medicate and non-medicated AN participants following oxytocin and placebo administration are presented in Supplementary table 4. P-values were corrected for multiple comparisons with the false discovery rate set at $q < 0.05$ (Benjamini and Hochberg, 1995). Following correction $p < 0.03$ was considered significant.

The mixed model revealed a significant drug x time interaction within the 500ms ITI blocks (Supplementary table 3). The post-hoc tests revealed that there was a significant difference between oxytocin and placebo at T2, after the smoothie challenge, ($Z = -2.34$, $p = 0.019$, 95% CI [-44.59, -3.97]), with all AN participants showing more AB away from food images following oxytocin administration. There

was no significant difference between oxytocin and placebo at T1 ($Z = 1.27$, $p = 0.203$, 95% CI [-5.95, 28.01]). There was also a significant difference between T1 and T2 only in the placebo condition ($Z = 3.66$, $p < 0.001$, 95% CI [11.28, 37.37]), with all AN participants showing more AB towards food images at T2 in the placebo condition. There were no significant differences between T1 and T2 in the oxytocin condition ($Z = -1.11$, $p = 0.268$, 95% CI [-30.42, 8.45]).

There was also a significant interaction between time and medication status within the 500ms ITI blocks (Supplementary table 4). The post-hoc tests revealed a significant difference between T1 at T2 within the medicated AN group ($Z = 3.38$, $p = 0.001$, 95% CI [9.34, 35.17]), with medicated AN participants showing more AB towards food images at T2, after the smoothie challenge. There were no significant differences between T1 at T2 within the non-medicated AN group ($Z = -1.11$, $p = 0.268$, 95% CI [-24.67, 6.85]). The post-hoc tests no significant differences between medicated and non-medicated AN participants at T1, before the smoothie challenge, ($Z = -2.19$, $p = 0.03$, 95% CI [-29.00, -1.59]), or at T2, after the smoothie challenge ($Z = 1.70$, $p = 0.089$, 95% CI [-2.39, 34.14]).

There were no significant differences between medicated and non-medicated AN participants in the 1250ms ITI blocks (Supplementary table 4).

Salivary cortisol and anti-depressants

Mean salivary cortisol levels are presented separately for the medicated and non-medicated AN participants in the oxytocin and placebo conditions in Supplementary

table 5. The mixed model revealed a significant main effect of medication status with those taking anti-depressants having lower salivary cortisol levels than non-medicated AN participants (Supplementary table 5). The main effect of drug also approached significance with oxytocin administration lowering salivary cortisol levels regardless of medication status.

Self-reported anxiety and anti-depressants

Mean salivary VAS anxiety ratings are presented separately for the medicated and non-medicated AN participants in the oxytocin and placebo conditions in Supplementary table 6. The mixed model revealed a significant main effect of medication status, with those taking anti-depressant reporting higher levels of anxiety than non-medicated AN participants (Supplementary table 6).

Additionally, there was a significant main effect of time, with all participants reporting lower anxiety before smoothie challenge than at baseline ($Z = -2.01$, $p = 0.044$, 95% CI [-0.12, -0.002]), but significantly higher anxiety after the smoothie challenge, than before ($Z = 3.87$, $p < 0.001$, 95% CI [0.05, 0.15]). There were no significant differences in self-reported anxiety at baseline and after smoothie challenge ($Z = 1.26$, $p = 0.206$, 95% CI [-0.02, 0.10]).

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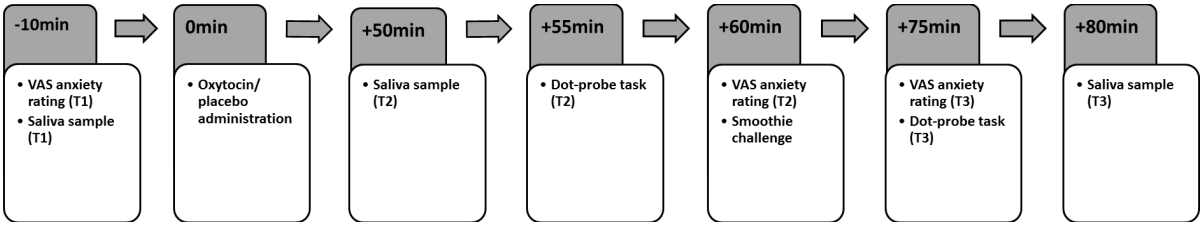
Appendix.

Please mark the following line at the point that most accurately reflects your current level of anxiety:

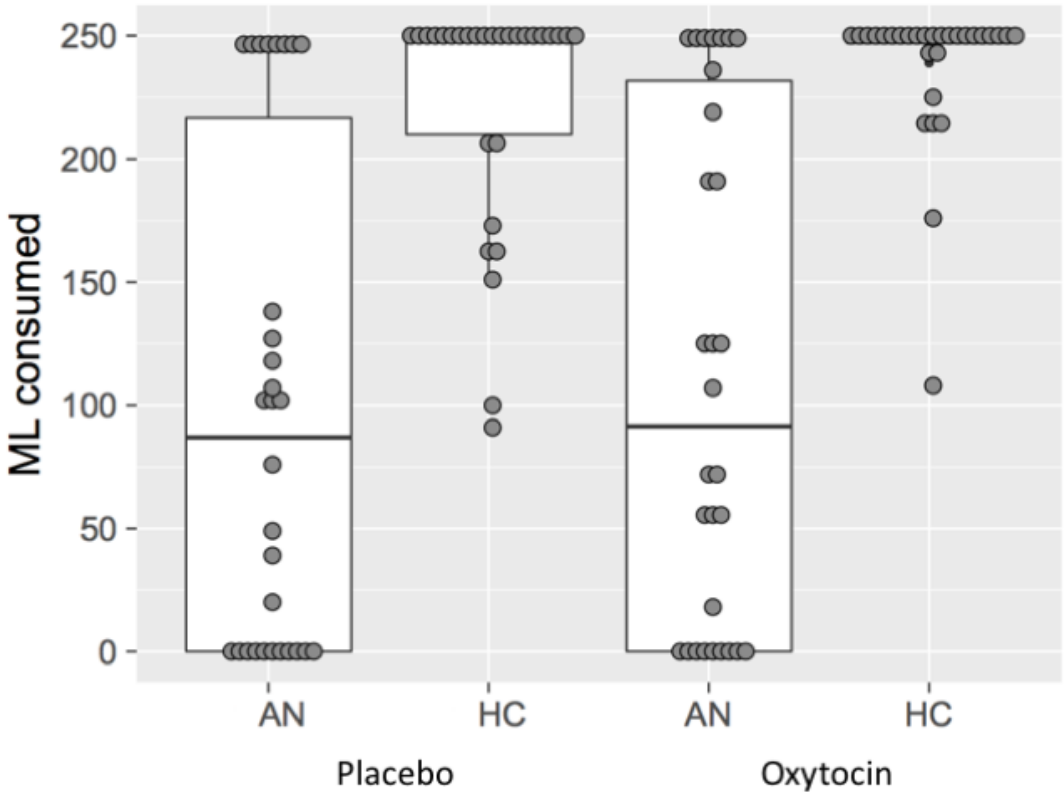
Not anxious at all _____ Extremely anxious

Supplementary Figure 1. Study flow chart. The grey boxes contain information regarding how many minutes before and after administration each task was delivered.



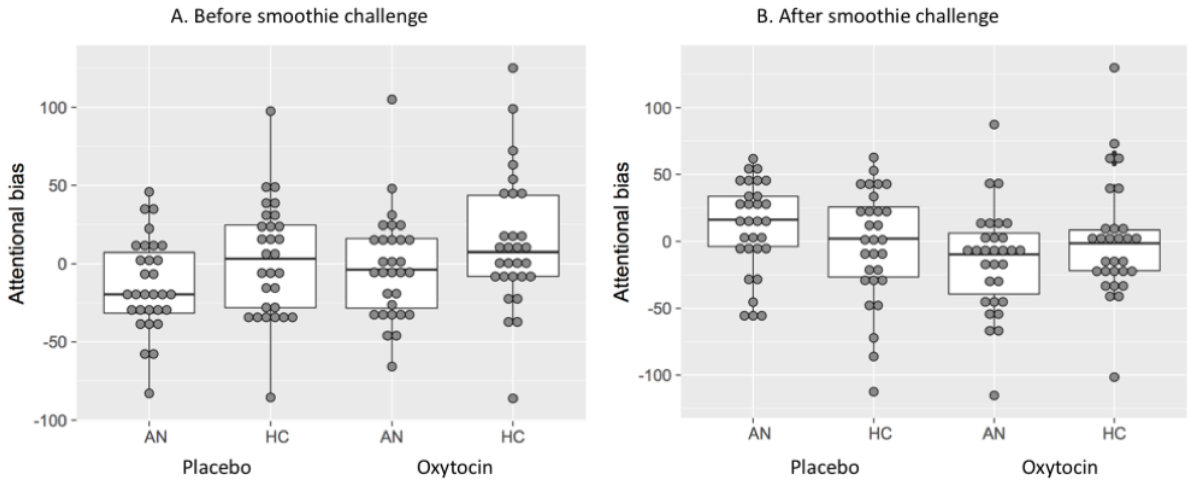
VAS = Visual analogue scale; T1 = before smoothie challenge; T2 = after smoothie challenge; min = minutes.

Supplementary Figure 2. Smoothie intake during the standard 15 minute smoothie challenge in ml.



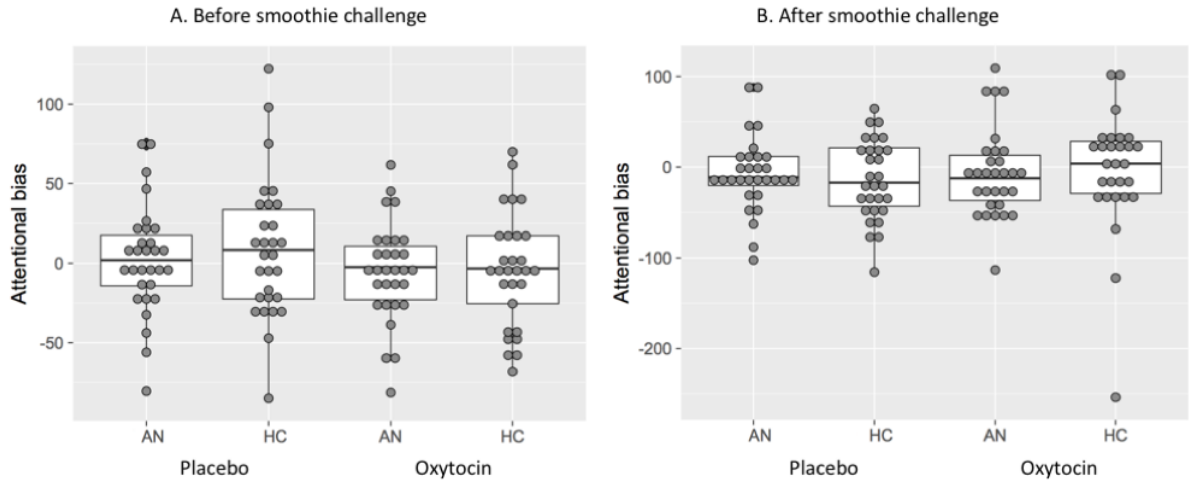
The grey circles represent individual data points, the box plots represent the median, 1st and 3rd quartiles along with minimum and maximum scores. AN = anorexia nervosa, HC = healthy comparison.

Supplementary Figure 3. Attentional bias towards food images in the 500ms ITI blocks in the AN and HC groups.



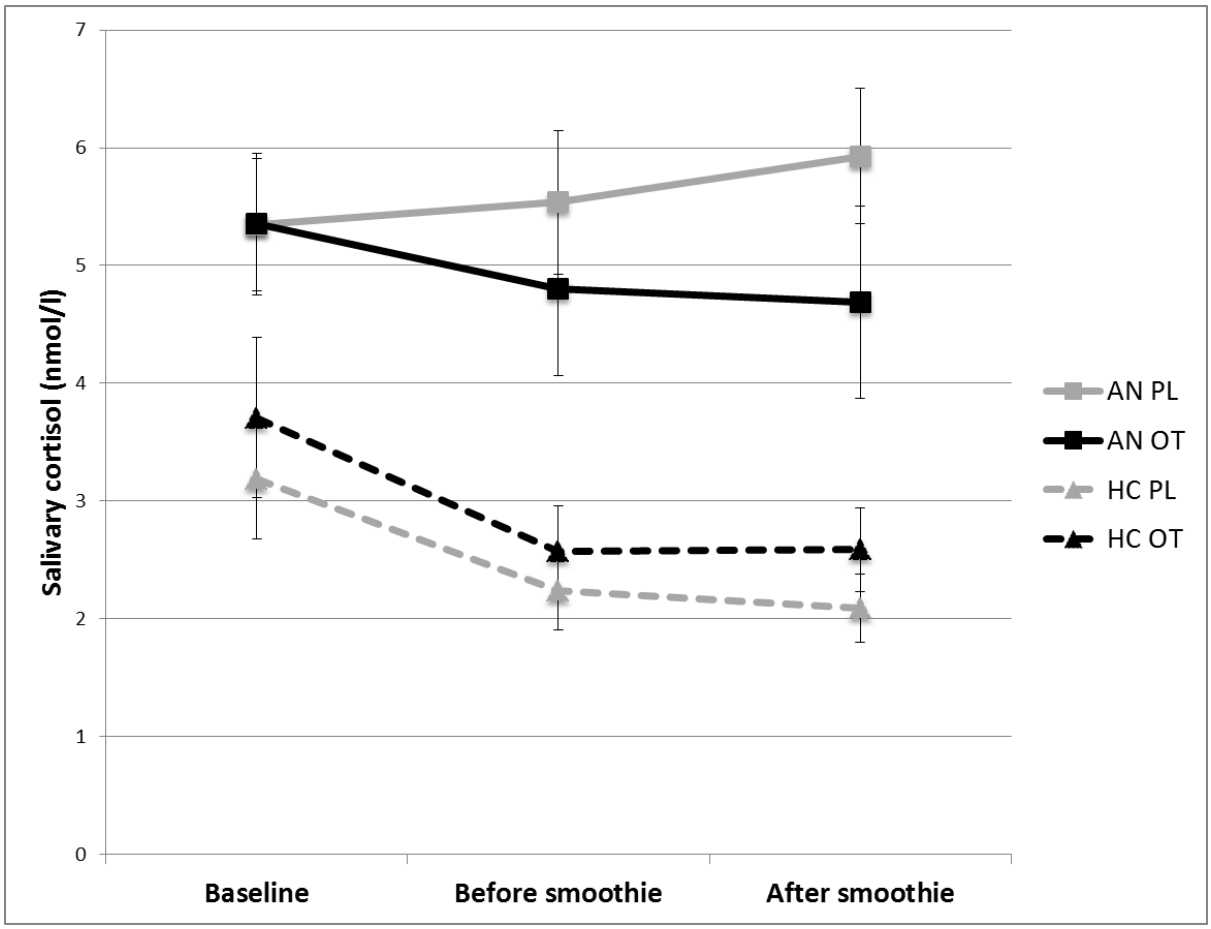
A.) AB scores for AN and HC groups in the placebo and oxytocin conditions at T1, before smoothie challenge, in 500ms ITI blocks. B.) AB scores for AN and HC groups in the placebo and oxytocin conditions at T2, after smoothie challenge, in 500ms ITI blocks. Positive scores indicate AB towards food images and negative scores indicate AB away from food images. The grey circles represent individual data points, the box plots represent the median, 1st and 3rd quartiles along with minimum and maximum scores. AN = anorexia nervosa, HC = healthy comparison, AB = attentional bias, ITI = inter-trial interval.

Supplementary Figure 4. Attentional bias towards food images in the 1250ms ITI blocks in the AN and HC groups



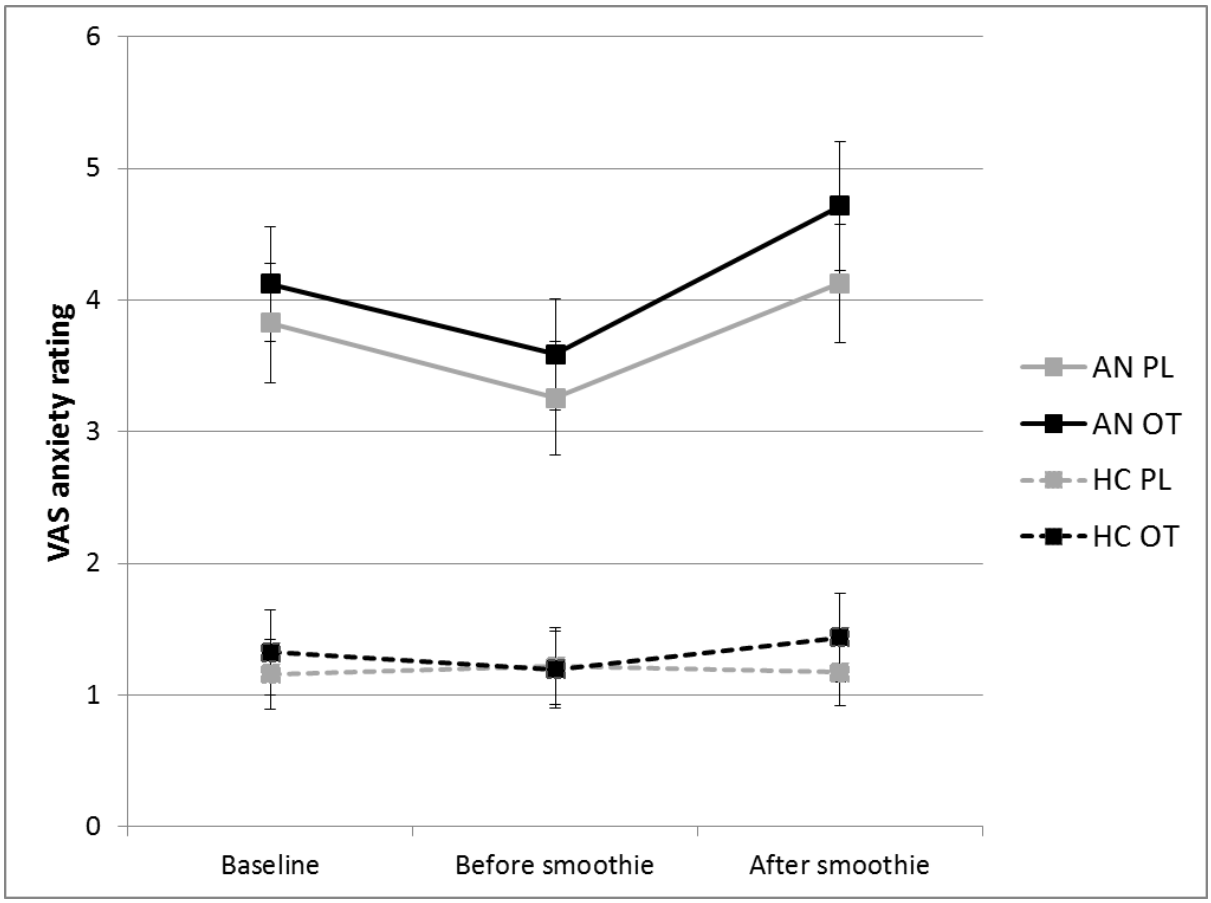
A.) AB scores for AN and HC groups in the placebo and oxytocin conditions at T1, before smoothie challenge, in 1250 ITI blocks. B.) AB scores for AN and HC groups in the placebo and oxytocin conditions at T2, after smoothie challenge, in 1250 ITI blocks. Positive scores indicate AB towards food images and negative scores indicate AB away from food images. The grey circles represent individual data points, the box plots represent the median, 1st and 3rd quartiles along with minimum and maximum scores. AN = anorexia nervosa, HC = healthy comparison, AB = attentional bias, ITI = inter-trial interval.

Supplementary Figure 5. Salivary cortisol levels in AN and HC participants.



The data represents mean raw salivary cortisol levels in nmol/l in AN group (solid lines) and HC group (dotted lines) at each time point as measured on the Salimetrics immunoassay kit. Error bars represent standard error of the mean. AN = anorexia nervosa, HC = healthy comparison, PL = placebo, OT = oxytocin.

Supplementary Figure 6. VAS anxiety ratings in AN and HC participants.



The data represents mean VAS anxiety ratings in AN group (solid lines) and HC group (dotted lines) at each time point. Error bars represent standard error of the mean. VAS = visual analogue scale, AN = anorexia nervosa, HC = healthy comparison, PL = placebo, OT = oxytocin.

Supplementary Table 1. AB towards food images before and after the test meal in the AN and HC groups in 1250ms ITI blocks.

		AN (N = 30)	HC (N = 29)	
Time	Drug	Mean (SD)	Mean (SD)	X^2 statistic, p value
Before smoothie challenge	Oxytocin	-5.06 (30.85)	8.66 (43.47)	Drug: $X^2 = 0.41$, p = 0.524
	Placebo	2.64 (34.08)	-4.53 (35.44)	Time: $X^2 = 1.97$, p = 0.160
				Group: $X^2 = 0.01$, 0.925
After smoothie challenge	Oxytocin	-6.78 (48.86)	-5.24 (67.38)	Drug x Time: $X^2 = 0.03$, p = 0.863
	Placebo	-8.13 (41.65)	-13.95 (44.29)	Drug x Group: $X^2 = 1.47$, p = 0.226
				Time x Group: $X^2 = 0.18$, p = 0.669
				Drug x Time x Group: $X^2 = 0.29$, p = 0.591

AN = anorexia nervosa, HC = healthy comparison, AB = Attentional bias, ITI = inter-trial; SD = standard deviation

Supplementary Table 2. VAS anxiety ratings at each time point in the AN and HC groups.

Time	Drug	AN (N = 30) Mean (SD)	HC (N = 29) Mean (SD)	X^2 statistic, p value
Baseline	Oxytocin	4.12 (2.36)	1.32 (1.75)	Drug: $X^2 = 2.11$, $p = 0.147$
	Placebo	3.83 (2.48)	1.16 (1.44)	Time: $X^2 = 13.27$, $p = 0.001$
Before smoothie challenge	Oxytocin	3.59 (2.31)	1.19 (1.58)	Group: $X^2 = 598.07$, $p < 0.001$
	Placebo	3.25 (2.35)	1.22 (1.57)	Drug x Time: $X^2 = 0.33$, $p = 0.848$
				Drug x Group: $X^2 = 1.31$, $p = 0.253$
After smoothie challenge	Oxytocin	4.71 (2.67)	1.44 (1.81)	Time x Group: $X^2 = 5.96$, $p = 0.051$
	Placebo	4.13 (2.48)	1.17 (1.38)	Drug x Time x Group: $X^2 = 0.14$, $p = 0.931$

AN = anorexia nervosa, HC = healthy comparison; SD = standard deviation

Supplementary Table 3. Clinical characteristics of medicated and non-medicated AN participants.

	Medicated AN (N = 15)	Non-medicated AN (N = 15)	Medicated AN vs. non-medicated AN	
	Median [Q1, Q3]	Median [Q1, Q3]	X^2 statistic, p value	Cramer's φ
BMI	17.63 [15.20, 18.51]	14.67 [14.37, 16.61]	X^2 = 2.13, p= 0.144	0.27
Age	25.00 [22.00, 27.00]	24.00 [21.00, 29.00]	X^2 = 0.00, p= 1.000	0.00
EDEQ total	4.49 [4.21, 5.50]	3.68 [2.66, 4.06]	X^2 = 19.27, p< 0.001	0.80
EDEQ restraint	4.40 [2.60, 5.20]	3.40 [1.40, 4.80]	X^2 = 0.07, p= 0.796	0.04
EDEQ weight concern	5.60 [3.80, 6.00]	3.00 [2.00, 4.60]	X^2 = 5.40, p= 0.020	0.42
EDEQ shape concern	5.88 [5.38, 6.00]	4.25 [2.75, 4.75]	X^2 = 19.27, p< 0.001	0.80
EDEQ eating concern	4.40 [3.20, 4.80]	3.6 [2.60, 4.40]	X^2 = 5.40, p= 0.020	0.42
DASS Total	72.00 [54.00, 88.00]	46.00 [26.00, 86.00]	X^2 = 6.67, p= 0.010	0.47
DASS Depression	30.00 [20.00, 40.00]	18.00 [10.00, 26.00]	X^2 = 6.67, p= 0.010	0.47
DASS Anxiety	18.00 [10.00, 26.00]	8.00 [2.00, 20.00]	X^2 = 4.29, p= 0.038	0.38
DASS Stress	26.00 [24.00, 30.00]	18.00 [14.00, 34.00]	X^2 = 0.27, p= 0.606	0.09

Q1 = 1st quartile, Q3 = 3rd quartile, AN = anorexia nervosa; HC = healthy control; EDEQ = Eating Disorders Examination Questionnaire; DASS = Depression, Anxiety, and Stress Scale

Supplementary Table 4. AB scores in medicated and non-medicated AN participants.

ITI block	Time	Drug	Medicated AN (N = 15) Mean (SD)	Non-medicated AN (N = 15) Mean (SD)	X^2 statistic, p value
500 ms ITI	Before smoothie challenge	Oxytocin	-11.03 (18.53)	5.71 (42.91)	Drug: $X^2 = 1.20$, p = 0.274
		Placebo	-20.62 (31.10)	-6.76 (26.54)	Time: $X^2 = 1.30$, p = 0.254
	After smoothie challenge	Oxytocin	-8.00 (43.06)	-19.28 (34.54)	Medication: $X^2 < 0.01$, p = 0.960
		Placebo	20.87 (29.04)	0.40 (37.23)	Drug x Time: $X^2 = 8.50$, p = 0.004
					Drug x Medication: $X^2 = 0.24$, p = 0.624
					Time x Medication: $X^2 = 7.06$, p = 0.008
					Drug x Time x Medication: $X^2 = 0.08$, p = 0.778
1250ms ITI	Before smoothie challenge	Oxytocin	-5.07 (28.70)	-5.06 (33.88)	Drug: $X^2 = 0.18$, p = 0.668
		Placebo	2.23 (18.57)	3.05 (45.40)	Time: $X^2 = 0.73$, p = 0.393
	After smoothie challenge	Oxytocin	-18.93 (52.55)	5.37 (43.20)	Medication: $X^2 = 1.37$, p = 0.241
		Placebo	-12.48 (22.52)	-3.79 (52.55)	Drug x Time: $X^2 = 0.41$, p = 0.524
					Drug x Medication: $X^2 = 0.26$, p = 0.611
					Time x Medication: $X^2 = 1.25$, p = 0.264
					Drug x Time x Medication: $X^2 = 0.31$, p = 0.579

AB = attentional bias; AN = anorexia nervosa; ITI = inter-trial interval; SD = standard deviation

Supplementary Table 5. Salivary cortisol at each time point in the medicated and non-medicated AN groups.

Time	Drug	Medicated AN (N = 15) Mean (SD)	Non-medicated AN (N = 15) Mean (SD)	X^2 statistic, p value
Baseline	Oxytocin	3.93 (3.43)	6.78 (1.80)	Drug: $X^2 = 3.41$, $p = 0.065$
	Placebo	4.27 (3.81)	6.42 (2.39)	Time: $X^2 = 0.22$, $p = 0.898$
Before smoothie challenge	Oxytocin	3.17 (2.56)	6.44 (3.30)	Medication: $X^2 = 1.87$, $p = 0.393$
	Placebo	3.99 (2.87)	7.08 (4.52)	Drug x Time: $X^2 = 64.19$, $p < 0.001$
After smoothie challenge	Oxytocin	3.33 (2.12)	6.04 (3.47)	Drug x Medication: $X^2 = 0.08$, $p = 0.783$
	Placebo	4.43 (4.16)	7.43 (4.42)	Time x Medication: $X^2 = 0.67$, $p = 0.714$
				Drug x Time x Medication: $X^2 = 0.30$, $p = 0.861$

AN = anorexia nervosa; SD = standard deviation

Supplementary Table 6. VAS anxiety ratings at each time point in the medicated and non-medicated AN groups.

Time	Drug	Medicated AN (N = 15) Mean (SD)	Non-medicated AN (N = 15) Mean (SD)	X^2 statistic, p value
Baseline	Oxytocin	4.78 (2.44)	3.46 (2.17)	Drug: $X^2 = 2.42$, $p = 0.120$
	Placebo	4.53 (2.40)	3.12 (2.44)	Time: $X^2 = 15.02$, $p = 0.0005$
Before smoothie challenge	Oxytocin	3.90 (2.30)	3.28 (2.35)	Medication: $X^2 = 0.02$, $p = 0.992$
	Placebo	3.72 (2.53)	2.78 (2.14)	Drug x Time: $X^2 = 25.88$, $p < 0.001$
After smoothie challenge	Oxytocin	5.52 (2.56)	3.91 (2.62)	Drug x Medication: $X^2 = 0.35$, $p = 0.554$
	Placebo	4.85 (2.54)	3.40 (2.26)	Time x Medication: $X^2 = 1.36$, $p = 0.507$
				Drug x Time x Medication: $X^2 = 0.11$, $p = 0.945$

AN = anorexia nervosa; SD = standard deviation

CHAPTER 6:

Effects of Intranasal Oxytocin on the Interpretation and Expression of Emotions in Anorexia Nervosa

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ORIGINAL ARTICLE

Effects of Intranasal Oxytocin on the Interpretation and Expression of Emotions in Anorexia Nervosa

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Altered social-emotional functioning is considered to play an important role in the development and maintenance of anorexia nervosa (AN). Recently, there has been increasing interest in investigating the role of intranasal oxytocin in social-emotional processing. The present study aimed to investigate the effects of intranasal oxytocin on the interpretation and expression of emotions among people with AN. Thirty women with AN and 29 age-matched healthy women took part in the present study, which used a double-blind, placebo-controlled, cross-over design. The participants received a single dose of 40 IU of intranasal oxytocin in one session and a placebo spray in the other. Fifteen minutes after administration, the participants completed the Reading the Mind in the Eyes Test to assess the interpretation of complex emotions and mental states followed by a video task, which assessed expressions of facial affect when they were viewing humorous and sad film clips. The intranasal oxytocin did not significantly influence the expression or interpretation of emotions in the AN or healthy comparison groups. The AN group expressed significantly less positive emotion, spent more time looking away and reported experiencing a significantly more negative affect in response to the film clips. The finding that intranasal oxytocin had little to no effect on the interpretation or expression of emotions in either group supports the notion that the effects of oxytocin on social-emotional processing are not straightforward and may depend on individual and environmental differences, as well as the emotion being processed. Replication of these findings is necessary to explore the effect of timing on the effects of oxytocin before firm conclusions can be drawn. Nonetheless, these findings add to the steady accumulation of evidence that people with AN have reduced emotional expression and avoidance of emotionally provoking stimuli.

Key words: oxytocin, anorexia nervosa, social-emotional functioning, facial expressions, positive affect, negative affect

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Current models of anorexia nervosa (AN) postulate that altered social cognition and difficulties in social-emotional functioning contribute to the development and maintenance of AN (1,2). Robust evidence from recent meta-analyses shows that people with AN have difficulties in a wide range of social-emotional domains, including the accurate interpretation and appropriate expression of emotions. Appropriate facial expression and mimicry increase prosocial behaviour and aid the accurate interpretation of emotions, whereas an inappropriate or blunted expression of emotions has been found to give rise to negative judgements and elicit a desire for increased social distance (3–5). In eating disorders, it has been suggested that problems in social communication contribute to the maintenance of disordered eating by causing interpersonal difficulties and increasing isolation (1,2). Thus, treatments targeting these interpersonal problems may be beneficial for people with AN.

Behavioural studies have demonstrated that people with AN have particular difficulties with accurately interpreting positive and negative emotions in faces, as well as tone of voice (6). People with AN

also have difficulties with accurately interpreting complex emotions and mental states in tasks such as the Reading the Mind in the Eyes Test (RMET) and other theory of mind tasks (6). Furthermore, a recent meta-analysis showed that people with AN, depression and autism display fewer positive and negative facial expressions when viewing positive and negative emotional film clips (7). Interestingly, people who have recovered from AN do not show similar difficulties in the interpretation and expression of emotions (6–9). Thus, these difficulties are possible secondary consequences of malnutrition; however, the exact mechanisms underlying difficulties in social-emotional processing in AN are still unclear.

The neuropeptide oxytocin is considered to pay a key role in social-emotional functioning (10,11). A recent review has documented a range of abnormalities in oxytocin functioning in acute AN (12). Additionally, another recent meta-analysis found that people with AN have significantly lower peripheral endogenous oxytocin levels than healthy individuals (13). Moreover, preliminary proof of concept studies have found that a single dose of intranasal oxytocin influences certain aspects of social-emotional processing in eating disorders (14,15). Among people with AN, a single dose of intranasal oxytocin attenuated attentional bias towards disgusted faces (15), although it did not increase sensitivity to recognise basic emotions, whereas, in people with bulimia nervosa and healthy comparison (HC) participants, intranasal oxytocin increased sensitivity to detect emotions (14). These mixed findings suggest that further exploration of the effects of a single dose of intranasal oxytocin on social-emotional functioning is still necessary.

A single dose of intranasal oxytocin has also been reported to improve the interpretation of complex emotional states in a number of different paradigms, including the RMET and the awareness of social inference test, among healthy individuals (16–18). Additionally, a single dose has been reported to further improve the interpretation of complex emotions among those healthy individuals who report difficulties in identifying and describing emotions (19). Among clinical populations, a single dose of intranasal oxytocin has been reported to improve the interpretation of complex emotions among people with schizophrenia and depression, comprising disorders characterised by difficulties in the interpretation of emotions (16,20). Thus, these findings suggest that a single dose of intranasal oxytocin may increase attention social-emotional cues particularly among those individuals who have difficulties with such social-emotional processes. However, to our knowledge, no studies to date have investigated the effects of a single dose of intranasal oxytocin on the interpretation of complex emotions in AN.

A single dose of intranasal oxytocin has also been found to increase emotional empathy and cooperation among healthy individuals particularly when social information regarding the other person was present (21,22). Additionally, recent studies have found that a single dose of intranasal oxytocin can increase cooperation and normalise the avoidant, flattened arousal responses to human social sounds in autism spectrum disorder (23,24). Thus, oxytocin may be a facilitator of social communication, including facial expression of emotion. Few studies thus far have investigated the effects of a single dose of oxytocin on emotion expression (25,26). Among healthy populations, a single dose of intranasal oxytocin has been reported to increase spontaneous facial mimicry in response to emotionally provoking videos, as well as increase facial expressivity during the instructed production of facial expressions (25,26). Among clinical populations, a single dose of intranasal oxytocin has been reported to increase facial expressivity in response to emotionally provoking images among people with schizophrenia and to reduce avoidant behaviours during a clinical interview in people with borderline personality disorder (27,28). However, to our knowledge, no studies to date have investigated the effects of intranasal oxytocin on evoked emotional facial expressions and the interpretation of complex emotions in AN.

The present study aimed to investigate the effects of a single dose of intranasal oxytocin on the interpretation and expression of emotions in people with AN and healthy control (HC) participants. Based on the previous work outlined above, we hypothesised that participants with AN would have anomalies in the interpretation and expression of emotions and that oxytocin administration would alleviate these anomalies. Additionally, we explored correlations between oxytocin-induced changes in the interpretation and expression of emotions and depression, autistic traits, and body mass index (BMI) within the AN group. Finally, we also explored differences in the interpretation and expression of emotions, as well as the effects of oxytocin, between AN participants who were taking antidepressants during the study and those AN participants who were free of psychotropic medication.

Materials and methods

Participants

Thirty women with AN were recruited to take part in the present study. The AN participants were recruited through South London and Maudsley National Health Service (NHS) Trust and eating disorder charity websites (BEAT https://www.b-eat.co.uk/ and Succeed www.succeed-foundation.org). All interested AN participants were included in the study if they were female, aged 16–65 years, met the DSM-5 criteria for AN assessed with the Structured Clinical Interview for DSM-5 (29) and did not meet any of the exclusion criteria for all participants. Fifteen of the AN participants were receiving psychotropic medication during the study. Twenty-nine HC women were recruited to take part in the study as a comparison group. The HC participants were recruited through King's College London circulars and notice boards in the local community. All HC participants were screened for past and current psychiatric disorders with the Structured Clinical Interview for DSM-5 (29) and excluded if they reported any current or history of mental health difficulties or suicidality. Additionally, participants were excluded if they met any of the overall exclusion criteria for all participants, which included being pregnant, a history of (or current) alcohol or drug abuse/misuse, hormonal disturbance not as a result of low weight among the AN participants, current impairments in cardiovascular functioning, and any regular medication excluding psychotropic medication among the AN participants. Five HC participants reported taking the contraceptive pill and were asked to take a break from the pill and wait for the onset of their next menstrual cycle before taking part. Prior to taking part in the study, all participants provided their written, informed consent. The study was approved by the National Health System Research Ethics Committee (14/LO/0128) and was conducted in accordance with the Helsinki Declaration of 1975 (revised in 2008).

The sample size was based on an *a priori* power analysis conducted with G*Power, which indicated that, altogether, 60 participants would be needed to have sufficient statistical power (0.8) to detect significant effects (30).

Self-report measures

The Eating Disorder Examination Questionnaire (EDEQ) was used to obtain an assessment of eating disorder psychopathology with the subscales: restraint, eating concern, weight concern and shape concern (31). The reliability of the EDEQ was high in the present study (Cronbach's $\alpha = 0.98$).

Assessments of depression, anxiety and stress were obtained using the Depression, Anxiety and Stress Scale (DASS) (32). The reliability of the DASS was high in the present study (Cronbach's $\alpha = 0.98$).

Autism Spectrum Quotient (AQ-10) is a short 10-item assessment of autistic traits with high positive predictive value at clinical cut-off of 6 (33). The reliability of the AQ-10 was acceptable in the present study (Cronbach's $\alpha = 0.70$).

Design and procedure

The present study used a within subjects, placebo-controlled, double-blind AB/BA cross-over design to explore the effects of intranasal oxytocin on evoked facial expressions in AN. The study consisted of two sessions. In one session, participants were asked to self-administer 10 puffs (five in each nostril) of a nasal spray containing 40 IU of synthetic oxytocin (Syntocinon; Novartis Pharmaceutical, Basel, Switzerland). In the other session, participants were asked to self-administer 10 puffs (five in each nostril) of a placebo (placebo) nasal spray. The dose was based on previous work investigating the effects of a single dose of intranasal oxytocin in eating disorders (14,15,34,35). The placebo nasal spray was custom made to be identical to the oxytocin spray minus the active ingredient. The order in which participants received oxytocin and placebo was counterbalanced and randomised. All HCs, and AN participants if menstruating, were tested during the first 1–10 days of the menstrual cycle. Additionally, the participants were asked not to consume any food 2 h before the sessions and to refrain from smoking or drinking alcoholic or caffeinated drinks 12 h before the sessions.

Fifteen minutes after administration, the participants were asked to complete a computerised version of the RMET (36) presented with EPRIME, version 2.0 (Psychology Software Tools, Inc., Sharpsburg, PA, USA). The RMET consists of a short practice trial and an experimental trial. In the practice trial, participants were presented with 10 images of eyes and were asked to identify whether the eyes were male or female. In the experimental trial, participants are presented 36 pictures of eyes (18 male and 18 female) depicting complex emotions. In this part of the task, participants are asked to identify the emotion that the eyes are conveying by selecting one of four options presented in each corner of the screen. The pictures were presented in a randomised order in each session. In the present study, participants were given infinite time to respond to each trial before moving onto the next. Participants' reaction times and accuracy were recorded.

Twenty-five minutes after administration, the participants were asked to watch two short video clips, each lasting 2–2.5 min, during which they were filmed. The two clips were chosen to evoke positive and negative emotions in the participants. The first film clip (henceforth Film 1) was a humorous scene from the film *Four Weddings and a Funeral* (1994), which has been previously successfully been used in a number of studies to evoke positive emotions (9,37–39). The second film clip (henceforth Film 2) was a sad scene chosen from the film *Shadowlands* (1999), which has also been previously successfully used to evoke negative emotions in the studies listed above (9,37–39). The same film clips were presented in both sessions in a fixed order with a short clip of computer simulated waves presented before each clip. This was performed to avoid carry-over effects from emotions experienced before the task started, as well as to avoid carry-over effects from the first film clip onto the second film clip.

After viewing each clip, participants were asked to rate how they were feeling on the Positive and Negative Affect Schedule (PANAS) (40). The PANAS consist of 20 emotion words and participants were asked to rate to what extent they were experiencing each emotion on a scale of 1 (very slightly or not at all) to 5 (extremely). The PANAS ratings were then analysed to establish participants self-reported positive and negative affect scores in response to Film 1 and Film 2.

Noldus FACEREADER

Noldus FACEREADER (Noldus Information Technology BV, Wageningen, The Netherlands) comprises facial expression analysis software that has been developed to identify six basic emotions: happiness, sadness, fear, anger, surprise and disgust. The software also detects neutral expressions. FACEREADER reports the intensity to which each of the six basic emotions are expressed in each frame on a scale of 0 to 1, where 0 indicates that the emotion is not present and 1 indicates that the emotion is fully present. FACEREADER has been validated using manual emotion coding systems and facial electromyography (41–43). The present study focused on exploring expressions of happiness and sadness.

Looking away

Looking away was analysed manually. The total number of seconds participants looked away from the film stimuli was recorded and analysed.

Statistical analysis

All data were analysed using STATA, version 14 (StataCorp, College Station, TX, USA). Differences in clinical and demographic self-report measures between the groups were analysed using a nonparametric median chi-squared test.

As a result of the highly skewed nature of the data, the PANAS ratings were transformed with square root transformation prior to analysis. Group differences and effects of oxytocin/placebo on the expression of emotions, PANAS ratings and looking away were explored with bootstrapped mixed linear models with 1000 bootstrap replications. Drug (oxytocin, placebo), Film (Film 1, Film 2), Group (AN, HC) and session (session 1, session 2) were entered as fixed effects with a random intercept. Technical difficulties with the video camera SD card led to loss of data from one AN participant and three HC participants.

As described above, group differences and the effect of drug on the interpretation of emotions on the RMET were examined using a bootstrapped mixed linear model (1000 replications) with Drug (oxytocin, placebo), Group (AN, HC) and Session (session 1, session 2) entered as fixed effects and a random intercept. As a result of the close proximity of the sessions (1–5 days apart), we included Session as a fixed effect to control for familiarity with the RMET and film stimuli. Significant interactions were further explored by investigating post-hoc contrasts and pairwise comparisons. We additionally conducted further post-hoc analysis investigating the effects of oxytocin on easy and difficult items between the two groups. We used a median split to divide items into easy (accuracy > 75.42%) and difficult (accuracy < 75.42%) groups based on total sample accuracy. These results are presented in the Supporting information (Doc S1, Table S2).

Additionally, we explored differences in the effects of intranasal oxytocin between AN participants taking antidepressants during the time of the study and those free of psychotropic medication in the above measures. As above, the effects of drug on the interpretation and expression of emotions, PANAS ratings and looking away were explored with similar $2 \times 2 \times 2 \times 2$ and $2 \times 2 \times 2$ bootstrapped mixed linear models with 1000 bootstrap replications. The results are presented in the Supporting information (Doc. S1, Table S3–S6, Figure S1–S2).

Within the AN group, relationships between oxytocin-induced changes in the interpretation and expression of emotions, and depression (DASS:

depression subscale), autistic features (AQ-10) and BMI were analysed with Spearman's rho correlation. Prior to correlation analysis, oxytocin-induced changes in the interpretation and expression of emotions were calculated by subtracting the interpretation accuracy and facial expression scores in the placebo condition from those in the oxytocin condition. Thus, positive scores indicated greater accuracy and facial expressivity in the oxytocin condition, whereas negative scores indicated greater accuracy and facial expressivity in the placebo session. The results are presented in the Supporting information (Doc S1, Table S6).

Results

Clinical and demographic characteristics

The clinical characteristics of the sample are presented in Table 1. The AN and HC groups were matched for age. The AN group had a significantly lower BMI than the HC group. Additionally, the AN group a reported significantly higher incidence of eating disorder psychopathology as measured on the EDEQ, and reported higher restraint, eating concern, shape concern and weight concern. Furthermore, the AN group scored higher on the DASS, reporting more depression, anxiety and stress than the HC group. Finally, there was a significant difference between the two groups on level of education, with HC participants reporting a higher level of education than the AN participants, 90% of whom reported having taken time off from education as a result of their illness.

There were no significant differences in age or BMI between those AN participants taking antidepressants during the study and those AN participants who were free of psychotropic medication (Table 1). The medicated AN participants did report a higher incidence of eating disorder symptomatology as measured on the EDEQ, scoring higher on the eating concern, weight concern and shape concern subscales than those free of medication. Additionally, the medicated AN participants scored higher on the DASS,

Table 1. Clinical and Demographic Sample Characteristics.

	AN (n = 30)	Medicated AN (n = 15)	Non-medicated AN (n = 15)	Medicated AN versus non-medicated AN χ^2, P value	HC (n = 29)	AN versus HC χ^2, P value
	Median (Q1, Q3)	Median (Q1, Q3)	Median (Q1, Q3)		Median (Q1, Q3)	
BMI	16.13 (14.56, 18.14)	17.63 (15.20, 18.51)	14.67 (14.37, 16.61)	2.13, 0.144	22.21 (20.78, 25.51)	35.55, < 0.001
Age	24.50 (22.00, 28.25)	25.00 (22.00, 27.00)	24.00 (21.00, 29.00)	0.00, 1.000	25.00 (23.00, 27.50)	0.02, 0.875
Level of education	3.00 (1.00, 3.00)	3.00 (1.00, 3.00)	3.00 (1.00, 3.50)	0.00, 1.000	3.00 (3.00, 4.00)	12.34, < 0.001
Time off education as a result of AN (years)	1.00 (0.69, 2.00)	1.50 (0.92, 2.50)	0.67 (0.25, 0.1.25)	2.17, 0.141	–	–
EDEQ total	4.13 (3.00, 5.13)	4.49 (4.21, 5.50)	3.68 (2.66, 4.06)	19.27, < 0.001	0.59 (0.34, 1.00)	35.27, < 0.001
EDEQ restraint	3.70 (2.60, 5.05)	4.40 (2.60, 5.20)	3.40 (1.40, 4.80)	0.07, 0.796	0.60 (0.00, 1.70)	20.79, < 0.001
EDEQ weight concern	4.20 (2.70, 5.65)	5.60 (3.80, 6.00)	3.00 (2.00, 4.60)	5.40, 0.020	0.60 (0.30, 1.40)	31.38, < 0.001
EDEQ shape concern	4.86 (3.56, 5.91)	5.88 (5.38, 6.00)	4.25 (2.75, 4.75)	19.27, < 0.001	0.75 (0.38, 1.38)	41.67, < 0.001
EDEQ eating concern	4.10 (2.90, 4.80)	4.40 (3.20, 4.80)	3.6 (2.60, 4.40)	5.40, 0.020	0.20 (0.00, 0.40)	41.67, < 0.001
DASS Total	63.00 (40.00, 86.00)	72.00 (54.00, 88.00)	46.00 (26.00, 86.00)	6.67, 0.010	4.00 (1.00, 10.00)	37.49, < 0.001
DASS Depression	21.00 (13.50, 32.00)	30.00 (20.00, 40.00)	18.00 (10.00, 26.00)	6.67, 0.010	0.00 (0.00, 2.00)	26.61, < 0.001
DASS Anxiety	12.00 (6.00, 21.00)	18.00 (10.00, 26.00)	8.00 (2.00, 20.00)	4.29, 0.038	0.00 (0.00, 2.00)	29.07, < 0.001
DASS Stress	25.00 (17.50, 31.00)	26.00 (24.00, 30.00)	18.00 (14.00, 34.00)	0.27, 0.606	2.00 (0.00, 7.00)	37.49, < 0.001
AQ	3.00 (4.00, 1.00)	2.00 (4.00, 1.00)	3.00 (4.00, 1.00)	0.17, 0.680	2.00 (3.00, 1.00)	1.38, 0.502

AN, anorexia nervosa; HC, healthy comparison; BMI, body mass index; EDEQ, Eating Disorder Examination Questionnaire; DASS, Depression, Anxiety and Stress Scale; AQ, Autism quotient; Level of education: 1 = A-level/National Vocational Qualification, 2 = Diploma, 3 = Undergraduate degree, 4 = Postgraduate degree.

reporting more depression and anxiety but not stress than the non-medicated AN participants (Table 1).

Performance on the RMET

Interpretation accuracy and reaction times (RT) in the AN and HC groups following both oxytocin and placebo administration are presented in Fig. 1(A,B). The mixed model revealed a significant difference between groups, with the AN participants being significantly more accurate than the HC participants (Table 2). There was also a significant effect of session, with all participants being significantly more accurate in session 2 than session 1 [Z = 2.39, P = 0.017, 95% confidence interval (CI) = 0.01–0.12]. There were no other significant effects or interactions influencing accuracy.

In terms of RT, the mixed model revealed a significant effect of session, with all participants responding faster in session 2 than session 1 (Z = −3.86, P < 0.001, 95% CI = −1211.80 to −395.67). There was also a significant Drug × Group × Session interaction (Table 2). The interaction was first explored by investigating the Drug × Group interaction within each session. The results revealed that there was a significant Drug × Group interaction in session 2 (χ^2 = 12.53, P = 0.0004) but not in session 1 (χ^2 = 2.70, P = 0.1006). The effect of drug was then investigated within each group in session 2. The results revealed that oxytocin led to a significantly slower RT in the AN group in (Z = 2.32, P = 0.020, 95% CI = 157.26–1871.20) and a faster RT in the HC group in session 2 (Z = −2.62, P = 0.009, 95% CI = −2059.48 to −269.17).

We also explored the Group × Session within placebo and oxytocin conditions. The post-hoc contrasts revealed a significant Group × Session interaction following both placebo (χ^2 = 6.83, P = 0.009) and oxytocin administration (χ^2 = 6.49, P = 0.011). The pairwise comparisons revealed that HC participants were significantly faster than the AN participants in session 2 (Z = −2.59, P = 0.010, 95% CI = −1912.21 to −263.49) but not in session 1 (Z = 1.13, P = 0.259, 95% CI = −431.42 to 1602.88) following oxytocin administration. Following placebo administration, the HC participants were significantly slower than the AN participants in session 2 (Z = 2.45, P = 0.014, 95% CI = 222.32–1986.10) but not in session 1 (Z = −1.19, P = 0.235, 95% CI = −1293.64–317.61) (Table 2).

Because 17 of the AN participants had completed the RMET before, we also explored differences between the groups on task performance without these participants to examine their impact. These findings are presented in the Supporting information (Doc S1). The results revealed that, although the AN participants were still on average more accurate than the HC participants, this difference no longer reached significance (see Supporting information, Table S1). As above, there was a significant effect of session suggesting that participants were more accurate in session 1 than in session 2.

Regarding RT, the results revealed a significant effect of session, with all participants being faster in session 2, and a significant Drug × Group interaction (see Supporting information, Table S1). Further exploration of the interaction revealed that the AN participants who had not completed the RMET before were significantly slower in the oxytocin than placebo condition (Z = 2.70, P = 0.007, 95% CI = 165.63–1045.27). A similar effect was not present in the HC group (Z = −0.56, P = 0.576, 95% CI = −503.51 to 280.00).

Expressions of happiness and sadness, and looking away

The intensity of expression of sadness and happiness in response to Film 1 and Film 2 following placebo and oxytocin administration is summarised for the AN and HC groups in Fig. 2(A,B) for Film 1 and Fig. 2(C,D) for Film 2. The P-value was adjusted for multiple post-hoc comparisons using the false discovery rate and was set at P < 0.003 (44). As expected, the mixed model revealed a significant effect of Film, with all participants across groups and sessions expressing more happiness when viewing Film 1 than Film 2. The results also revealed a significant effect of Group, with HC participants expressing more positive facial expressions than AN participants across films and sessions (Table 3). The model also revealed a significant Film × Group and Drug × Film × Group × Session interactions.

The Film × Group interaction was investigated further by exploring the difference between the groups at the two levels of Film. The post-hoc test revealed that the HC participants expressed significantly more happiness than the AN participants when viewing Film 1 (Z = 4.29, P < 0.0001, 95% CI = 0.08–0.21). There were no

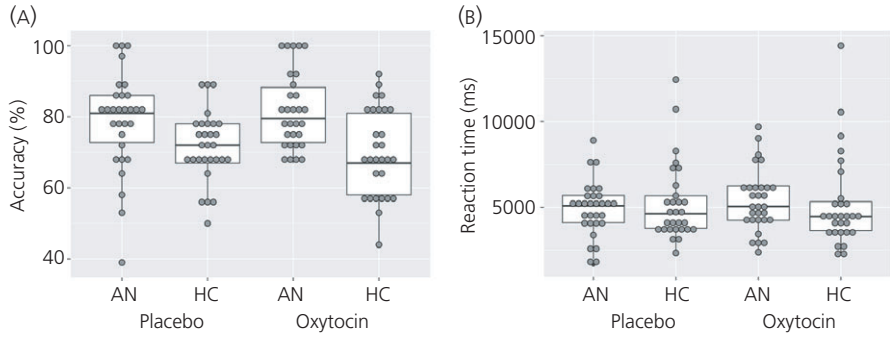

Fig. 1. Percentage accuracy (A) and reaction times (B) on the Reading the Mind in the Eyes (RMET) in the anorexia nervosa (AN) and healthy comparison (HC) groups in the oxytocin and placebo conditions. Grey dots represent individual data points; the box plot represents the median, interquartile range, and the maximum and minimum of the data. RT, reaction time; ms, milliseconds.

Table 2. Performance on the Reading the Mind in the Eyes Test (RMET) in Anorexia Nervosa (AN) and Healthy Comparison (HC) Groups Following Oxytocin and Placebo.

	Drug	AN (n = 30), mean ± SD	HC (n = 29), mean ± SD	χ^2, P value
Accuracy (%)	Oxytocin	81.77 ± 10.67	69.83 ± 12.35	Drug: 0.27, 0.604
	Placebo	78.83 ± 13.74	71.79 ± 9.63	Session: 5.69, 0.017
				Group: 31.25, < 0.001
				Drug × Session: 2.86, 0.091
				Drug × Group: 1.33, 0.250
				Session × Group: 0.71, 0.400
				Drug × Session × Group: 0.72, 0.397
RT	Oxytocin	5379.72 ± 1802.67	5158.24 ± 2671.37	Drug: 0.65, 0.420
	Placebo	4929.32 ± 1600.89	5241.59 ± 2282.45	Session: 14.90, < 0.001
				Group: 0.01, 0.909
				Drug × Session: 0.97, 0.324
				Drug × Group: 1.84, 0.175
				Session × Group: 0.01, 0.920
				Drug × Session × Group: 11.31, 0.001

RT, reaction time.

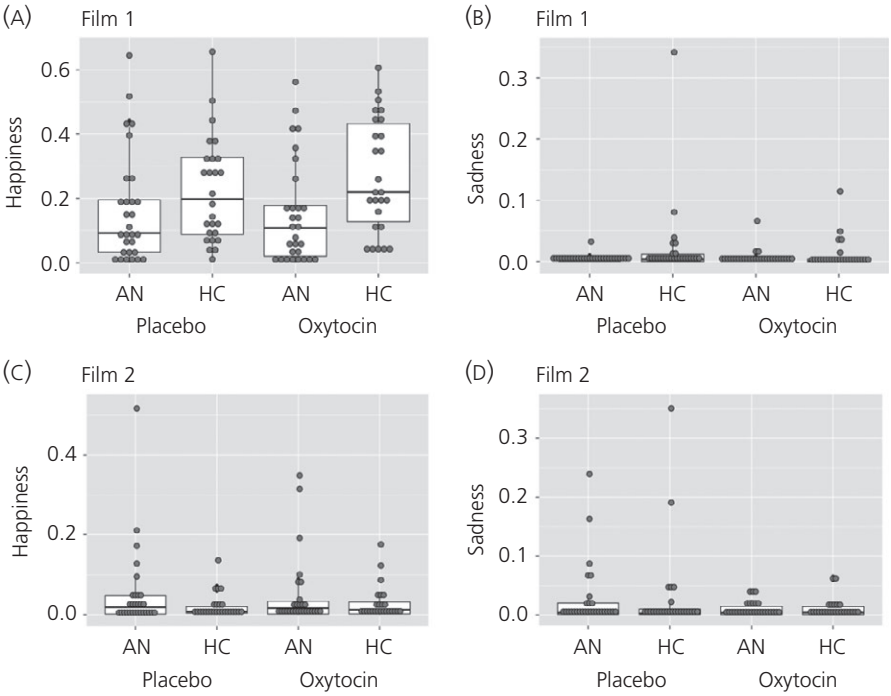

Fig. 2. Intensity of expressions of sadness and happiness in response to Film 1 and Film 2 in the anorexia nervosa (AN) and healthy comparison (HC) groups in oxytocin and placebo conditions. (A) Expressions of happiness in response Film 1, (B) expressions of sadness in response to Film 1, (C) expressions of happiness in response to Film 2, (D) expressions of sadness in response to Film 2. Grey dots represent individual data points; the box plot represents the median, interquartile range, and the maximum and minimum of the data. Film 1, humorous film clip; Film 2, sad film clip.

significant differences between the groups in expressions of happiness when viewing Film 2 (Z = −1.34, P = 0.181, 95% CI = −0.10–0.02).

The four-way interaction was first investigated by exploring the Drug × Film × Group interaction within session 1 (χ^2 = 3.84, P = 0.05) and session 2 (χ^2 = 1.02, P = 0.314), which did not yield significant results. Next, the Drug × Film × Session interaction was explored within the HC (χ^2 = 2.23, P = 0.135) and AN groups (χ^2 = 1.85, P = 0.173), which did not yield significant results. Finally, the Drug × Session × Group interaction was explored within Film 1 (χ^2 = 4.06, P = 0.044) and Film 2 (χ^2 = 1.07, P = 0.301), which also did not yield significant results. There were no other significant effects or interactions influencing expressions of happiness.

Regarding the expression of sadness, as above, the mixed model revealed a significant effect of Film, with all participants across

Table 3. Expressions of Happiness and Sadness, and Looking Away in Anorexia Nervosa (AN) and Healthy Comparison (HC) Groups During Film 1 and Film 2.

	Film	Drug	AN (n = 30), mean ± SD	HC (n = 29), mean ± SD	χ^2, P value
Expressions of happiness	Film 1	Oxytocin	0.15 ± 0.16	0.27 ± 0.18	Drug: 0.47, 0.494
		Placebo	0.16 ± 0.17	0.22 ± 0.16	Film: 134.66, < 0.001
	Film 2	Oxytocin	0.05 ± 0.09	0.03 ± 0.04	Group: 5.81, 0.016
		Placebo	0.05 ± 0.10	0.02 ± 0.03	Session: 0.20, 0.592
					Drug × Film: 0.12, 0.727
					Drug × Group: 1.63, 0.202
					Film × Group: 14.29, < 0.001
					Drug × Session: 0.05, 0.818
					Film × Session: 2.08, 0.149
					Group × Session: 1.11, 0.293
					Drug × Film × Group: 0.29, 0.592
					Drug × Film × Session: 0.03, 0.857
					Drug × Group × Session: 0.94, 0.857
					Film × Group × Session: 0.01, 0.905
					Drug × Film × Group × Session: 4.07, 0.044
Expressions of sadness	Film 1	Oxytocin	0.01 ± 0.01	0.01 ± 0.02	Drug: 3.62, 0.057
		Placebo	0.004 ± 0.006	0.02 ± 0.07	Film: 7.19, 0.007
	Film 2	Oxytocin	0.01 ± 0.01	0.01 ± 0.02	Group: 0.61, 0.436
		Placebo	0.03 ± 0.05	0.03 ± 0.08	Session: 1.73, 0.188
					Drug × Film: 0.76, 0.384
					Drug × Group: 0.73, 0.392
					Film × Group: 1.30, 0.254
					Drug × Session: 0.06, 0.801
					Film × Session: 0.57, 0.451
					Group × Session: 0.34, 0.559
					Drug × Film × Group: 1.13, 0.289
					Drug × Film × Session: 0.64, 0.422
					Drug × Group × Session: 0.71, 0.398
					Film × Group × Session: 0.23, 0.631
					Drug × Film × Group × Session: 0.48, 0.488
Looking away (s)	Film 1	Oxytocin	1.28 ± 2.49	0.23 ± 0.51	Drug: < 0.01, 0.948
		Placebo	1.00 ± 1.93	0.31 ± 0.74	Film: 8.58, 0.003
	Film 2	Oxytocin	2.72 ± 3.72	0.65 ± 2.08	Group: 18.05, < 0.001
		Placebo	2.38 ± 4.69	1.08 ± 2.35	Session: 7.97, 0.005
					Drug × Film: 0.07, 0.797
					Drug × Group: 0.72, 0.397
					Film × Group: 1.87, 0.172
					Drug × Session: 3.11, 0.078
					Film × Session: 0.09, 0.762
					Group × Session: 3.31, 0.069
					Drug × Film × Group: 0.12, 0.728
					Drug × Film × Session: 0.09, 0.767
					Drug × Group × Session: 1.98, 0.160
					Film × Group × Session: 0.04, 0.841
					Drug × Film × Group × Session: 2.59, 0.107

Film 1, humorous film clip; Film 2, sad film clip.

groups and sessions expressing more sadness when viewing Film 2 than Film 1. There were no other significant effects or interactions influencing expressions of sadness.

The total time (s) that participants looked away from the film stimuli is presented in Table 3. The mixed model revealed a significant effect of group, with AN participants looking away more than HC participants. There was also a significant effect of film and session, with all participants spending more time looking away from Film 2 than Film 1, and looking away more during session 2 than session 1. There were no other significant effects or interactions (Table 3).

Subjective PANAS ratings

Ratings of subjective positive and negative affect following Film 1 and Film 2 in the AN and HC groups are presented in Table 4. The P-value was adjusted for multiple post-hoc comparisons using

the false discovery rate and was set at $P < 0.004$ (44). The mixed model exploring ratings of positive affect on the PANAS revealed a significant effect of group in ratings of positive affect, with the HC participants reporting a more subjective positive affect relative to the AN participants (Table 4). There was also a significant effect of Film, with all participants reporting more positive affects following Film 1 than Film 2, and a significant effect of session, with participants reporting a more positive affect in session 1 than session 2.

The mixed model exploring ratings of subjective positive affect also revealed significant Film × Session and Drug × Film × Session interactions (Table 4). The three-way interaction was first explored by investigating the Drug × Film interaction in session 1 and session 2, which revealed no significant interactions (session 1: $\chi^2 = 2.53$, $P = 0.112$; session 2: $\chi^2 = 4.04$, $P = 0.044$). The three-way interaction was then explored by investigating the Session × Drug interaction in the two levels of film, which revealed no significant interactions (Film 1: $\chi^2 = 5.24$, $P = 0.023$; Film 2: $\chi^2 = 1.34$, $P = 0.247$). Finally, the three-way interaction was explored by investigating the Film × Session interaction at the two levels of drug, which revealed a significant interaction in the oxytocin ($\chi^2 = 11.19$, $P = 0.001$) but not placebo session ($\chi^2 < 0.01$, $P = 0.980$). Further pairwise comparison revealed that participants who received oxytocin in the first session and those who received it in the second session reported a more subjective positive affect in response to Film 1 than Film 2 (session 1; $Z = -7.55$, $P < 0.001$, 95% CI = −11.42 to −6.71; session 2: $Z = -2.16$, $P = 0.031$, 95% CI = −5.00 to −0.24). There was also a significant Group × Session interaction (Table 4), with HC participants reporting a significantly more positive affect in session 1 than 2 ($Z = -3.84$, $P < 0.001$, 95% CI = −4.21 to −1.36). There was no significant differences between the session within the AN group ($Z = -0.84$, $P = 0.399$, 95% CI = −2.14 to 0.86).

The mixed model exploring ratings of negative affect revealed a significant effect of Film, with all participants reporting significantly more negative affect in response to Film 2 than Film 1. There was also a significant difference between groups in ratings of negative affect, with AN participants reporting a more negative affect than the HC participants (Table 4).

Table 4. Positive and Negative Affect Schedule (PANAS) Rating Following Film 1 and Film 2 in Anorexia Nervosa (AN) and Healthy Comparison (HC) Groups.

PANAS	Film	Drug	AN (n = 30), mean ± SD	HC (n = 29), mean ± SD	χ^2, P value
Positive affect	Film 1	Oxytocin	11.00 ± 4.67	14.36 ± 7.16	Drug: 1.30, 0.254
		Placebo	11.72 ± 5.71	15.84 ± 6.04	Film: 131.20, < 0.001
	Film 2	Oxytocin	5.21 ± 4.29	8.72 ± 7.27	Group: 58.09,< 0.001
		Placebo	5.57 ± 4.61	9.60 ± 6.51	Session: 10.66, 0.001
					Drug × Film: 0.05, 0.829
					Drug × Group: 0.29, 0.587
					Film × Group: 0.02, 0.879
					Drug × Session: 1.09, 0.297
					Film × Session: 6.38, 0.012
					Group × Session: 4.10, 0.043
					Drug × Film × Group: 0.07, 0.789
					Drug × Film × Session: 4.54, 0.032
					Drug × Group × Session: 0.02, 0.882
					Film × Group × Session: 2.53, 0.111
					Drug × Film × Group × Session: 0.43, 0.512
Negative affect	Film 1	Oxytocin	5.07 ± 6.08	0.88 ± 1.69	Drug: 0.64, 0.425
		Placebo	4.28 ± 4.57	0.88 ± 1.45	Film: 33.10, < 0.001
	Film 2	Oxytocin	8.82 ± 6.78	3.68 ± 5.37	Group: 69.99, < 0.001
		Placebo	8.57 ± 5.49	3.08 ± 3.30	Session: 0.05, 0.824
					Drug × Film: < 0.01, 0.950
					Drug × Group: 0.05, 0.820
					Film × Group: 1.32, 0.251
					Drug × Session: 1.14, 0.285
					Film × Session: 0.29, 0.592
					Group × Session: 0.41, 0.521
					Drug × Film × Group: 0.28, 0.599
					Drug × Film × Session: 0.10, 0.750
					Drug × Group × Session: 1.54, 0.215
					Film × Group × Session: 0.03, 0.872
					Drug × Film × Group × Session: 0.18, 0.673

Film 1, humorous film clip; Film 2, sad film clip.

Discussion

The present study aimed to investigate the effects of a single dose of intranasal oxytocin on the interpretation and expression of emotions in people with AN and HC participants. By contrast to what was hypothesised based on the previous literature, oxytocin administration did not have a significant impact on the interpretation or expression of emotions in either group. However, as hypothesised, the present findings replicated previous work demonstrating that, relative to HC, people with AN display a less positive facial affect, spend more time looking away from the emotional stimuli, and report a less subjective positive affect when viewing emotional film stimuli. Surprisingly, the AN participants were significantly more accurate with respect to interpreting complex emotions in the RMET than the HC participants. Finally, we found that AN participants who were taking antidepressants during the study expressed a less positive facial affect when viewing the humorous film and were less accurate with respect to interpreting complex emotions relative to non-medicated AN participants.

By contrast to what was hypothesised, we did not find significant effects of intranasal oxytocin on the expression of facial affect. These findings were somewhat surprising considering that previous studies have reported a significant effect of oxytocin on expression of emotions among healthy and clinical populations (25–27). The discrepancy could arise from the fact that previous studies have largely utilised different kinds of emotionally provoking stimuli, including threatening social and nonsocial stimuli (25–27). Indeed, Korb and Malsert (25) found no changes in the expression of facial affect in response to positive facial expressions, although they did find changes in frowning in response to angry faces. Furthermore, intranasal oxytocin may differentially influence spontaneous facial expression, instructed production of facial expression (26) and facial mimicry during an emotion identification task (25). However, in a recent meta-analysis and systematic review conducted by our group, we found that oxytocin did not have a significant effect on emotion expression (J. Leppanen, K. W. Ng, K. Tchanturia and J. Treasure, unpublished data).

Additionally, we found no significant effects of oxytocin administration on the accuracy of complex emotion interpretation in the AN or HC groups. In part, these findings support a recent proof of concept study, which found that intranasal oxytocin did not improve sensitivity to recognise basic emotions in AN (14). However, the previous study used a different task to assess interpretation of emotions and found that intranasal oxytocin improved emotion recognition sensitivity in HC participants and people with bulimia nervosa (14). Similarly, other previous studies have reported that a single dose of intranasal oxytocin significantly improved recognition of complex emotions on the RMET among healthy individuals (17,18). However, recently, there have also been some failed replications of these findings suggesting that the effects of intranasal oxytocin on social-emotional processing are likely to more complex than previously anticipated (45–47). Furthermore, in our recent meta-analysis and systematic review, we found that, when fourteen previous studies were pooled together, a single dose on intranasal oxytocin did not have a significant impact on any theory of mind measures, including RMET (J. Leppanen, K. W. Ng, K. Tchanturia and J. Treasure, unpublished data). However, in a simpler test of recognition of basic emotions, a single dose of oxytocin improved recognition of fear and increased sensitivity to recognise anger in healthy individuals only (J. Leppanen, K. W. Ng, K. Tchanturia and J. Treasure, unpublished data).

Indeed, a systematic review has reported that almost half of studies investigating the effects of intranasal oxytocin on social-emotional processing did not report significant effects of oxytocin. Furthermore, where significant effects were found, 63% of the time, the effects were moderated by environmental factors or individual differences (10). Additionally, a more recent systematic review has suggested that the varied effects of oxytocin on social-emotional processing may be moderated by social boundaries, such that oxytocin not only increases social-affiliative behaviour within an 'in-group', but also increases distrust and desire to punish strangers and 'out-group' members (48). This notion is supported by a recent study reporting that oxytocin-induced noncooperation in the prisoner's dilemma, a trust game, was influenced by fear (49). It was suggested that oxytocin may have increased sensitivity fear-inducing social stimuli leading to noncooperation (49). Thus, it may be that the previously seen effects of oxytocin on social-emotional processing are a by-product of its effects on feelings of belongingness. Further exploration of the effects of oxytocin on expression of emotions in different social boundary conditions may be of interest with respect to evaluating its therapeutic potential.

When viewing the humorous film clip, the AN participants expressed significantly less positive facial affect than the HC participants, although there were no significant differences between the groups when viewing the sad film clip. Relative to the HC group, the AN participants also spend more time looking away from both emotional film clips, and reported experiencing a less positive affect and a more negative affect in response to the humorous and sad film clips. The present findings add to the steady accumulation of evidence indicating that the AN group has difficulties expressing positive emotions and also has a tendency to avoid emotional stimuli (7,37–39,50). Furthermore, previous studies have shown that, relative to HC women, adolescents and adult women with AN do not display more negative facial expressions in response to negative film stimuli despite reporting a more negative affect (38,51). Taken together, these findings suggest that there may be a general disconnect between subjective experience and external display of emotions and a tendency to avoid emotional engagement. Such difficulties have important affective and social consequences (4,52) and are thus important targets for interventions.

By contrast to what we expected, AN participants were significantly more accurate than the HC participants with respect to interpreting complex emotions in the RMET. This finding is in direct contrast with previous work showing that AN participants have difficulties with accurately interpreting complex emotions in the RMET (53–55). However, upon closer inspection, on average, the AN participants in the present study did not appear to outperform those in some previous studies (53,55). The HC participants, on the other hand, were less accurate than those in previous case–control studies and large sample validation studies (53–57). Although, it is

difficult to know what led to these unexpected findings, it is unlikely to be a result of the HC group having general difficulties with understanding the task. Had that been the case, we would have expected there to be significant differences between the groups in accuracy and response times, which did not occur in the present study. Additionally, the majority of the AN participants (57%) were more familiar with the RMET than HC participants, having completed it before as part of other studies, and some of them performed unexpectedly well on the task, with accuracy percentages of up to 100%. Indeed, once these participants were removed, the differences between the AN and HC groups did not reach significance. However, there still remained a small difference between the groups, with AN participants being more accurate.

Interestingly, we also found significant differences in accuracy and reaction times between sessions 1 and 2 on the RMET across groups, with all participants becoming more accurate and faster in session 2 even though no feedback on performance was given. These findings appear surprising considering that previous test–retest reliability studies have found no such effects in the RMET among healthy populations (58,59). However, it is of note that, in these studies, a minimum of 2 weeks fell between the first administration of the task and the retest (58,59), whereas, in the present study, the two sessions were only 1–5 days apart. Other studies that have used shorter intervals between test and retest, ranging from 1 h to 1 week, have found improvements in accuracy and response times in a range of social-emotional tasks (60,61). Furthermore, a few studies have found implicit learning effects over the course an emotion recognition task in healthy and other clinical populations, including people with depression and borderline personality disorder (62,63). Therefore, it is possible that, because the two sessions in the present study were so close, participants became familiarised with the stimuli, leading to a significant improvement in task performance.

The present study also explored differences between participants taking antidepressants and those free of psychotropic medication. As might be expected, those taking antidepressants reported significantly more eating disorder psychopathology, depression and anxiety than those free of psychotropic medication, suggesting that medicated AN participants were more severe. However, when these confounding factors were accounted for, the medicated AN participants remained significantly less accurate with respect to interpreting emotions, and were less expressive and reported a less positive affect when watching the humorous Film 1 than the non-medicated AN group. Although, it is possible that additional confounders were not accounted for, a recent study investigating facial expressivity among people with AN also reported a reduced expression of positive facial affect among AN participants taking antidepressants (9). Further exploration of the effects of antidepressants on social-emotional processing in people with acute AN may be of interest.

Clinical implications

The present findings add further evidence to the wealth of research suggesting that people with AN may have a general disconnect between subjective experience and expression of emotions (38,51). Appropriate expression of emotions is one of the corner stones of social interaction and a blunted facial affect can have profound affective and social consequences (4,52). Behavioural studies have found that inhibiting the expression of facial affect in response to emotionally provoking stimuli is associated with increases in blood pressure and reductions in subjective enjoyment (52,64). Additionally, when viewing videos of people displaying either appropriate, inappropriate or blunted reactions to emotional stimuli, participants rated those people who displayed a blunted response to positive emotional stimuli more negatively and expressed a greater desire for increased social distance from these individuals (4). Thus, these problems with social communication may contribute to the elevated negative mood, isolation and loneliness that are common features of AN (65).

Limitations

The main limitation of the present study was the relatively short interval between the administration of oxytocin and the first task. However, there are previous studies that have used a similar short interval between administration and the task and have reported significant differences in social-emotional processing between oxytocin and placebo sessions (66). However, it is not possible to determine whether the lack of oxytocin-induced effects in the RMET in the present study was not at least partly a result of this short interval. Still, previous work investigating the temporal profile of another neuropeptide similar to oxytocin (i.e. vasopressin) has found that significant effects occur within 10 min of administration (67). Nonetheless, replication of the present findings with a longer interval between administration and tasks is necessary.

Although the sad film has been successfully used in many previous studies (37–39), in the present study, we found that the majority of the participants were not familiar with the film *Shadowlands* (1999) and did not understand it. Thus, most of the participants were not sufficiently affected by the film and did not express a sufficient negative facial affect to be detected by FACEREADER, which provides a mean valence score for the duration of the film. If participants expressed a negative facial affect on only couple of occasions during the film but were otherwise neutral during the majority of the film, those short expressions would be averaged out in the mean valence score. A previous study used clips from different films tailored specifically for younger participants and found robust results showing reduced facial expressivity in young people with AN when viewing both happy and sad film clips (51). Future research may benefit from tailoring the clips for the participant group to ensure they are sufficiently familiar with the story to engage with the clips.

Compared to previous work (37,38,53,54), the present study employed a modified procedure in which the experimenter stayed in the room during all tasks. Although this allowed the experimenter to tackle some practical difficulties (e.g. a participant moving outside of camera range or watching the clips in a different order), it may have led the participants to feel less able to engage and become involved with the film clips during the film task. Future

studies may benefit from finding alternative ways to combat non-compliance, at the same time as giving participants sufficient privacy to engage with the films.

Finally, the present study did not assess participants IQ, which can impact task performance. This is a limitation when comparing the performance of the two groups on the RMET, which requires an understanding of adjectives describing complex emotions. Although this did not directly impact the investigation of the effects of oxytocin in the cross-over study, future work should conduct a full IQ assessment when attempting to compare the performance of two or more groups on a similar neuropsychological test.

Conclusions

The present study aimed to investigate the effects of a single dose of intranasal oxytocin on difficulties in the interpretation and expression of emotions in AN. The findings revealed that oxytocin did not significantly influence the interpretation of complex emotions or facial expressivity in response to positive or sad emotional stimuli in AN. These findings are supported by recent work showing that intranasal oxytocin does not improve emotion recognition sensitivity in people with AN and selectively improves the expression of negative emotions in response to threatening stimuli (14,25–27). However, replication of these findings with different intervals between oxytocin administration and the task is necessary before firm conclusions can be drawn. Taken together, these findings suggest that there may be anomalies present in social-emotional processing in people with AN that are not resolved with a single dose of intranasal oxytocin.

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Conflicts of interest

The authors declare that there they have no conflicts of interest.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. Performance on the Reading the Mind in the Eyes (RMET) in the medicated and non-medicated anorexia nervosa (AN) groups in oxytocin and placebo conditions.

Fig. S2. Intensity of expressions of sadness and happiness in response to Film 1 and Film 2 in the medicated and non-medicated anorexia nervosa (AN) groups in oxytocin and placebo conditions.

Doc. S1. Supplementary materials.

Table S1. Performance on the Reading the Mind in the Eyes (RMET) in anorexia nervosa (AN) and healthy comparison (HC) participants who had not completed the task before following oxytocin and placebo.

Table S2. Accuracy on the Reading the Mind in the Eyes (RMET) by item difficulty following oxytocin and placebo administration in anorexia nervosa (AN) and healthy comparison (HC) groups.

Table S3. Performance on the Reading the Mind in the Eyes (RMET) in medicated and non-medicated anorexia nervosa (AN) participants.

Table S4. Expressions of happiness and sadness, and looking away in medicated and non-medicated anorexia nervosa (AN) participants during Film 1 and Film 2.

Table S5. PANAS ratings in medicated and non-medicated anorexia nervosa (AN) participants following Film 1 and Film 2.

Table S6. Correlations between oxytocin-induced changes in interpretation and expression of emotions and autistic traits, body mass index (BMI) and depression.

Supplementary materials

1.1 RMET analysis by item difficulty

Drug (oxytocin, placebo) x group (AN, HC) x category (easy, difficult) mixed linear model with 1000 bootstrap repetitions revealed a significant effect of group, with AN participants being significantly more accurate than HCs (Supplementary table 2). As expected there was additionally a significant effect of category with all participants being more accurate in the easy trials than difficult trials. There were no other significant effects or interactions.

1.2 Performance on the RMET in medicated and non-medicated AN participants

Interpretation accuracy and RT in the medicated and non-medicated AN groups following both oxytocin and placebo administration are presented in Supplementary Figure 1. Because the medicated and non-medicated AN groups differed significantly on the EDEQ and DASS an composite score was created adding the total scores together. This composite score was then used to control for differences arising from self-reported psychopathology in all analysis investigating differences between medicated and non-medicated participants.

The results revealed a significant effect of medication on accuracy, with the non-medicated AN participants being significantly more accurate interpreting complex emotions that the medicated AN participants (Supplementary Table 3). There were no other significant effects or interactions influencing accuracy or RT.

1.3 Expressions of happiness and sadness, and looking away in medicated and non-medicated AN participants

Intensity of expressions of sadness and happiness in response to Film 1 and Film 2 following placebo and oxytocin administration are summarised for the medicated and non-medicated AN participants in Supplementary Figure 2 (Film 1: A, B; Film 2: C, D). As above all analyses were conducted controlling for self-reported psychopathology.

As expected, the mixed model revealed only a significant effect of film on expressions of sadness, with all AN participants expressing more sadness while viewing Film 2 than Film 1 (Supplementary Table 4). There was no significant effect of medication status or any other significant main effects not interactions influencing expressions of sadness.

As above, the results also revealed a significant effect of film, with all AN participants expressing more happiness in response to Film 1 than Film 2. Despite controlling for self-reported psychopathology, there was also a significant effects of medication status, with non-medicated AN participants expressing significantly more happiness across films and condition than the medicated AN participants.

Additionally, the results revealed a significant film x medication status interaction. Post-hoc pairwise comparisons revealed that non-medicated AN participants expressed significantly more happiness than medicated AN participants while viewing Film 1 ($Z = -4.70$, $p < 0.001$, 95% CI [-0.31, -0.13]), but not Film 2 ($Z = -0.38$, $p = 0.701$, 95% CI [-0.10, 0.07]).

Total time medicated and non-medicated AN participants spent looking away from the film stimuli is presented in Supplementary Table 2. There was a significant effect of film with all AN participants spending more time looking away from Film 2 than Film 1. There were no other significant effects of interactions influencing looking away.

1.4 PANAS ratings in medicated and non-medicated AN participants

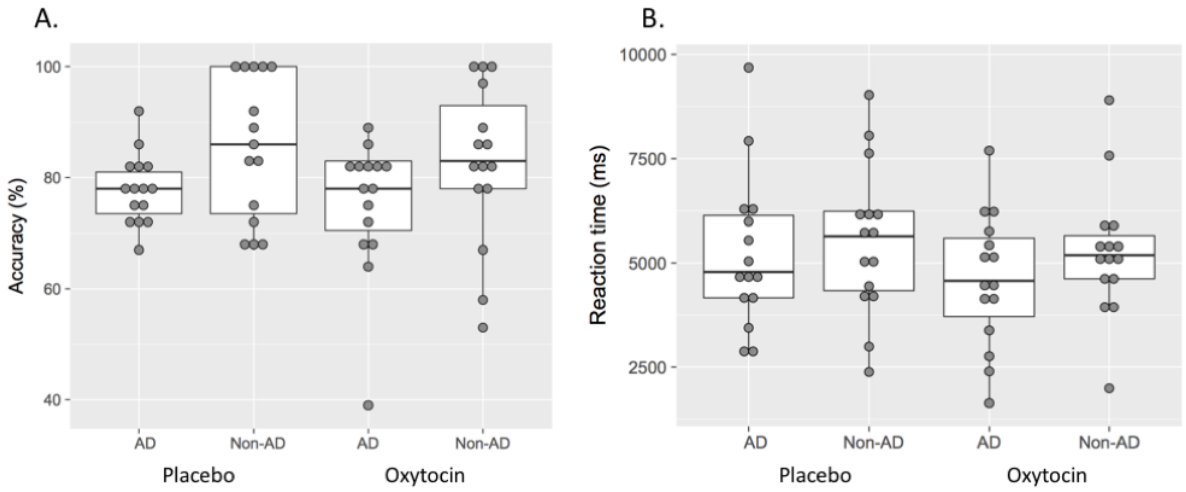
Both the mixed model exploring ratings of positive affect and that exploring ratings of negative affect found a significant effect of Film (Supplementary Table 5). As above all analyses were conducted controlling for self-reported psychopathology. All AN participants reporting more positive affect in response to Film 1 than Film 2, and more negative affect in response to Film 2 than Film 1.

The mixed model exploring ratings of positive affect revealed a significant effect of group, with non-medicated AN participants reporting significantly more positive affect relative to medicated AN participants (Supplementary Table 5).

1.5 Correlations between oxytocin-induced changes in interpretation and expression of emotions and depression, autistic traits, and BMI,

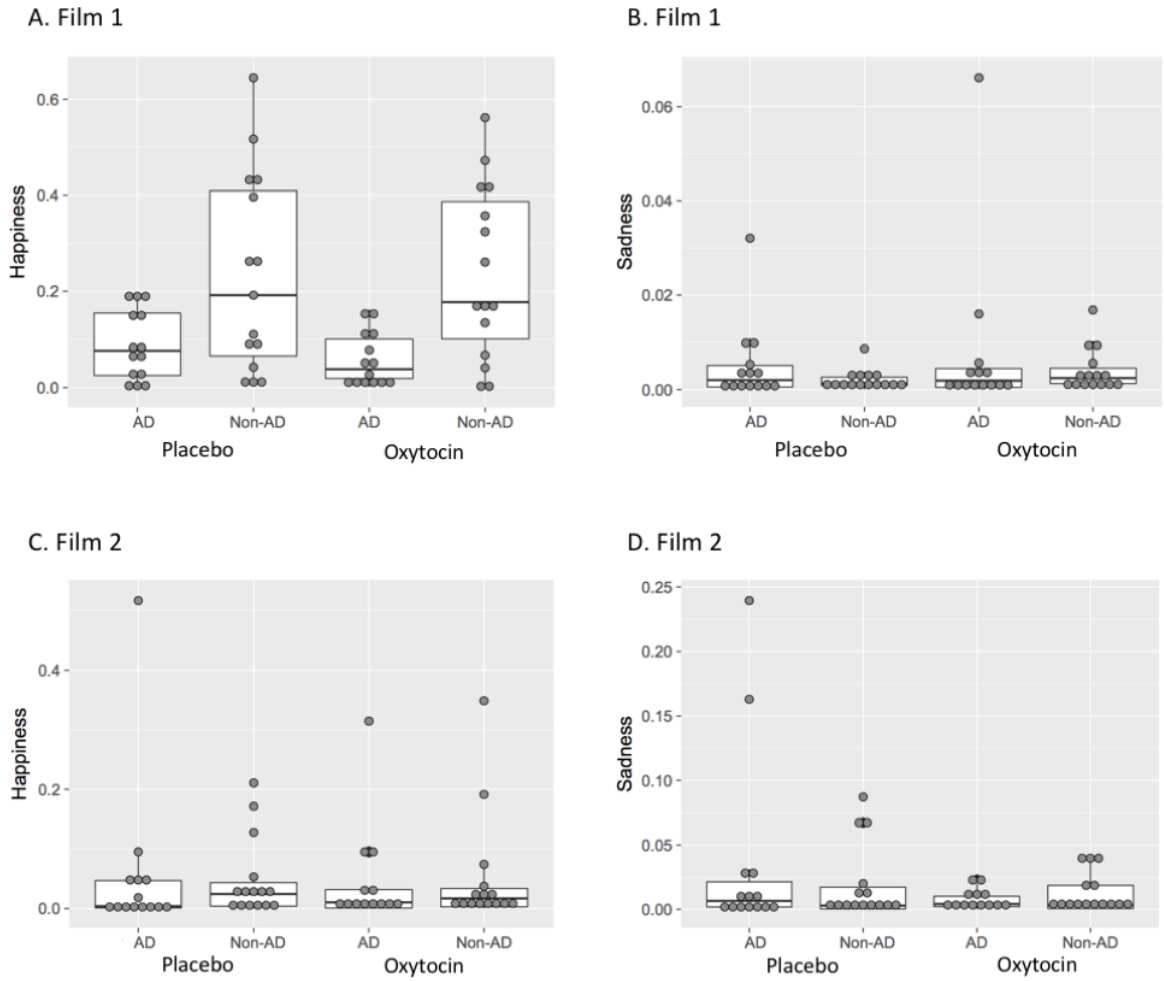
There were no significant correlations between interpretation or expression of emotions and autistic traits, BMI, or depression within the AN group (Supplementary Table 6).

Supplementary Figure 1. Performance on the RMET in the medicated (AD) and non-medicated (Non-AD) AN groups in the oxytocin and placebo conditions.



A.) Percentage accuracy on the RMET. B.) reaction times on the RMET in milliseconds (ms). The grey dots represent individual data points, the box plots represent the median, 1st and 3rd quartiles along with minimum and maximum scores. AN = anorexia nervosa; RMET= Reading the Mind in the Eyes Task; RT = reaction time; ms = milliseconds.

Supplementary Figure 2. Intensity of expressions of sadness and happiness in response to Film 1 and Film 2 in the medicated (AD) and non-medicated (Non-AD) AN groups in oxytocin and placebo conditions.



A.) Expressions of happiness during the humorous film clip (Film 1). B.) Expressions of sadness during the humorous film clip (Film 1). C.) Expressions of happiness during the sad film clip (Film 2). D.) Expressions of sadness during the sad film clip (Film 2). The grey dots represent individual data points, the box plots represent the median, 1st and 3rd quartiles along with minimum and maximum scores. AD = taking anti-depressants; Non-AD = no taking anti-depressants; Film 1 = humorous film clip; Film 2 = sad film clip.

Supplementary Table 1. Performance on the RMET in AN and HC participants who had not completed the task before following oxytocin and placebo.

		AN (N = 13)	HC (N = 29)	
	Drug	Mean (SD)	Mean (SD)	X^2 statistic, p value
Accuracy (%)	Oxytocin	77.08 (7.88)	69.83 (12.35)	Drug: $X^2 = 2.00$, p = 0.157
	Placebo	74.85 (5.29)	71.79 (9.63)	Session: $X^2 = 4.09$, p = 0.043
				Group: $X^2 = 2.13$, p = 0.145
				Drug x Session: $X^2 = 1.33$, p = 0.249
				Drug x Group: $X^2 = 0.14$, p = 0.706
				Session x Group: $X^2 = 0.28$, p = 0.597
				Drug x Session x Group: $X^2 = 0.28$, p = 0.597
RT	Oxytocin	5266.14 (1962.82)	5158.24 (2671.37)	Drug: $X^2 = 2.53$, p = 0.112
	Placebo	4592.68 (1116.02)	5241.59 (2282.45)	Session: $X^2 = 53.55$, p < 0.001
				Group: $X^2 = 0.20$, p = 0.651
				Drug x Session: $X^2 = 3.04$, p = 0.081
				Drug x Group: $X^2 = 5.57$, p = 0.018
				Session x Group: $X^2 = 2.69$, p = 0.101
				Drug x Session x Group: $X^2 = 0.02$, p = 0.893

AN = anorexia nervosa, HC = healthy comparison, RMET = Reading the Mind in the Eyes; RT = reaction time, SD = standard deviation

Supplementary Table 2. Accuracy on the RMET by item difficulty following oxytocin and placebo administration in AN and HC groups.

			AN (N = 30)	HC (N = 29)	
	Category	Drug	Mean (SD)	Mean (SD)	X^2 statistic, p value
Accuracy (%)	Easy	Oxytocin	0.90 (8.30)	77.20 (13.47)	Drug: $X^2 = 0.09$, p = 0.763
		Placebo	85.74 (14.27)	81.42 (12.88)	Category: $X^2 = 119.13$, p < 0.001
	Difficult	Oxytocin	73.52 (16.03)	62.26 (14.75)	Group: $X^2 = 42.11$, p < 0.001
		Placebo	71.85 (15.64)	62.07 (13.20)	Drug x Category: $X^2 = 0.09$, p = 0.759
					Drug x Group: $X^2 = 2.47$, p = 0.116
					Category x Group: $X^2 = 0.40$, p = 0.526
					Drug x Category x Group: $X^2 = 1.38$, p = 0.24

AN = anorexia nervosa, HC = healthy comparison, RMET = Reading the Mind in the Eyes, SD = standard deviation

CHAPTER 7:

Meta-analytic review of the effects of a single dose of intranasal oxytocin on threat processing in humans

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Review article

Meta-analytic review of the effects of a single dose of intranasal oxytocin on threat processing in humans

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ABSTRACT

Background: Heightened threat sensitivity is a transdiagnostic feature in several psychiatric disorders. The neuropeptide oxytocin has been shown to reduce fear related behaviours and facilitated fear extinction in animals. These findings have led to increasing interest to explore the effects of intranasal oxytocin on threat processing in humans.

Methods: The review included 26 studies (N = 1173), nine of which included clinical populations (N = 234). The clinical groups included were people with borderline personality disorder (BPD), anorexia nervosa, bulimia nervosa, depression, anxiety, and alcohol dependence disorder. We examined the effects of a single dose of intranasal oxytocin on startle response, attentional responses, and behavioural responses to threat.

Results: A single dose of intranasal oxytocin significantly increased the physiological startle response to threat in healthy people with a small effect size. However, oxytocin did not have significant effects on attentional bias towards social or disorder-specific threat, fixation towards threatening stimuli among healthy or clinical populations, or on threat related behavioural approach or avoidance responses.

Limitations: No studies investigated the effects of oxytocin on the startle response to threat among clinical populations. Additionally, only one of the reviewed studies had sufficient power to detect at least a moderate effect of oxytocin according to our criterion.

Discussion: The synthesis of literature suggest that oxytocin may influence the salience of threatening stimuli among healthy individuals, increasing the startle response to threat. It would be of interest to investigate the effects of oxytocin on the startle response to threat among clinical populations.

1. Introduction

Threat processing is vital for the survival of an organism or species (Öhman, 2005, 2007). Potential threats are rapidly recognised and activate a number of subcortical structures including the amygdala and the hypothalamic-pituitary-adrenal (HPA) axis, which initiate protective fear responses (Öhman, 2005). Physiological responses, such as increased skin conductance, behavioural approach and avoidance responses, such as fight or flight responses, and attentional responses, such as active attending to the source of fear also occur (Misslin, 2003). However, hypersensitivity of this system and maladaptive fear learning can have negative consequences (Ozawa and Johansen, 2014). Such maladaptive processes are believed to contribute to the development and maintenance of number of psychiatric disorders, such as eating disorders (ED), anxiety disorders, obsessive-compulsive disorder (OCD), and post-traumatic stress disorder (PTSD) (Britton et al., 2011; Ozawa and Johansen, 2014; Strober et al., 2007).

Behavioural studies have demonstrated that people with PTSD, OCD, and ED show anomalies in physiological responses to threat. For example, there is an exaggerated physiological startle response to anticipation and viewing of disorder-specific, potentially threatening stimuli, such as images of trauma, contamination, or food (Altman et al., 2013; Mauler et al., 2006; Pitman et al., 2012; Simon et al., 2013). Additionally, a number of reviews have reported atypical attentional bias towards disorder specific stimuli (Bar-Haim et al., 2007; Brooks et al., 2011; Cisler and Koster, 2010). Furthermore, disorder specific stimuli have been found to elicit negative facial expressions and subjective feelings of fear and disgust (Broderick et al., 2013; Soussignan

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et al., 2011; Uher et al., 2004). Taken together these findings suggest that treatments targeting this hypersensitivity and maladaptive fear learning may be of benefit in these disorders.

The neuropeptide, oxytocin, has been found to play an important regulatory role in human and animal studies (Neumann and Slattery, 2016; Onaka et al., 2012; Zheng et al., 2010). Animal studies have demonstrated that oxytocin plays an important role in lowering the physiological stress and anxiety response by activating a negative feedback loop (Onaka et al., 2012; Zheng et al., 2010). Elevated oxytocin secretion from the paraventricular nucleus of the hypothalamus following a stressful event is believed to lead to increased availability of gamma-aminobutyric acid (GABA), which in turn is believed to inhibit the HPA axis and amygdala activation (Onaka et al., 2012; Zheng et al., 2010). Indeed, a recent study found that marmoset monkeys treated with an oxytocin receptor antagonist had elevated glucocorticoid levels in response to the stressor and showed a greater tendency to engage in maladaptive fear-related behaviour such as isolation (Cavanaugh et al., 2016). These findings have sparked increasing interest in exploring the role of oxytocin in threat processing, particularly in fear responses and extinction learning.

Preclinical studies have demonstrated that exogenous intravenous and intranasal oxytocin can influence fear related responses in rodents and monkeys respectively, reducing hiding behaviour during an open field test and attentional bias towards threatening facial expressions (Parr et al., 2013; Rotzinger et al., 2010). Additionally, a recent systematic review explored the role of the oxytocin system in fear extinction in rodents (Neumann and Slattery, 2016). The findings revealed that oxytocin reduced fear related behaviours and facilitated fear extinction in rodents, particularly when the exogenous oxytocin or oxytocin agonists were administered to the infralimbic cortex (Neumann and Slattery, 2016). The infralimbic cortex has inhibitory projections to a number of subcortical regions in the rodent brain including the amygdala and activation of this region has been documented to be associated with fear extinction in rodents and humans (Quirk and Beer, 2006). These findings suggest that oxytocin may reduce fear and facilitate extinction possibly by reducing stress and anxiety around the source of potential threat. Thus, these findings have led to increasing interest to investigate the role oxytocin plays in threat processing and fear extinction in humans as well as its therapeutic potential in disorders characterised by elevated threat sensitivity and resistance to natural extinction.

A few studies have begun to explore the effects of intranasal oxytocin on threat processing and the physiological stress and anxiety response in humans. For example a recent meta-analysis found that a single dose of intranasal oxytocin lowered cortisol in clinical populations characterised by dysregulation of the HPA axis, but did not significantly influence the cortisol response to stressful stimuli in healthy individuals (Cardoso et al., 2014). Since many psychiatric disorders, including ED, PTSD, and anxiety disorders, are characterised by dysregulation of the HPA axis (Connan et al., 2007; Ehlert et al., 2001; Lo Sauro et al., 2008), there has been increasing interest in further exploration of the potential anti-stress and anxiolytic effects of intranasal oxytocin. To our knowledge, no systematic reviews have thus far investigated the effects of single dose of intranasal oxytocin on different aspects of threat processing in healthy and clinical populations more broadly.

The aim of the current systematic review and meta-analyses was to build on previous literature and synthesise studies examining the effects of a single dose of intranasal oxytocin on threat processing among in humans. The objective was to investigate the effects of oxytocin on the physiological startle response, behavioural approach and avoidance responses, and attentional responses, including attentional bias and fixation, towards generally threatening stimuli as well as towards disorder related threatening stimuli among clinical populations. Based on findings outlined above, we hypothesised that a single dose of intranasal oxytocin would reduce these anomalous threat responses in clinical populations characterised by elevated threat sensitivity.

2. Methodology

2.1. Literature search

In accordance with the PRISMA guidelines (Moher et al., 2009), electronic databases, including OVID (PsycINFO, PsycARTICLES, Medline, ARGIS), Web of Knowledge core collection, and Pubmed, were searched April 2017. The search terms included *oxytocin AND (threat OR fear OR anxiety OR avoidance OR attention OR bias OR startle OR approach OR fixation OR gaze)*. Furthermore, bibliographies of included studies were inspected to look for further studies not yielded by the initial search.

2.2. Inclusion criteria

In order to be included in the systematic reviews and meta-analyses studies were required to meet the following inclusion criteria: 1) investigate physiological response, behavioural approach and avoidance response, attention, or fixation towards threatening or feared stimuli; 2) investigate the effects of a single dose of intranasal oxytocin on these measures; 3) compare the effects of intranasal oxytocin against intranasal placebo; 4) include healthy adult participants and/or adult clinical populations; 5) random allocation of participants to receive intranasal oxytocin or placebo in studies using between subjects design; 6) randomisation of treatment order in studies using within subjects design; 7) be published in English in a peer reviewed journal. In order to reduce heterogeneity, long trials in which participants received more than one dose of oxytocin or in which effects of oxytocin were investigated the following day or later were not included.

2.3. Study selection

The search flow diagram is presented in Fig. 1. The literature search and initial screening based on title and abstract was conducted by the first author. The full text articles identified in the initial search and screening were examined for eligibility by two authors in conjunction (J.L. and K.W.N.). Studies were then included in the systematic review and meta-analyses if both authors agreed they met the inclusion criteria. If there was any uncertainty regarding eligibility of a paper it was referred to the rest of team for further discussion.

2.4. Data collection and synthesis

We conducted three separate meta-analyses investigating the effects of a single dose of intranasal oxytocin on the physiological response, behavioural approach and avoidance response, attention, and fixation towards threatening stimuli. To conduct these meta-analyses information regarding means, standard deviations, and sample size were extracted from the included articles. Where standard errors of the mean (SE) were reported instead of standard deviations, these were converted with the following formula: $SD = SE^{*}\sqrt{N}$. Fourteen studies did report the data in the article or in Supplementary Materials, or reported the data in figures only. In order to acquire the required data, the corresponding authors of these papers were contacted by one of the authors. The relevant data was obtained from the following studies via personal communication: Acheson et al. (2013); Bertsch et al. (2013); Eckstein et al. (2015); Eckstein et al. (2016); Hubble et al. (2017); Kim et al., In prep; Leknes et al. (2013); Preckel et al. (2014); Striepens et al. (2012). Despite contacting corresponding authors, we were unable to gain access to the relevant data from eight studies.

In addition to healthy individuals, clinical populations included in the study consisted of people with anorexia nervosa (AN), bulimia nervosa (BN), depression, and BPD. Information regarding participants' age, dose of oxytocin (in international units), the type of task used, the

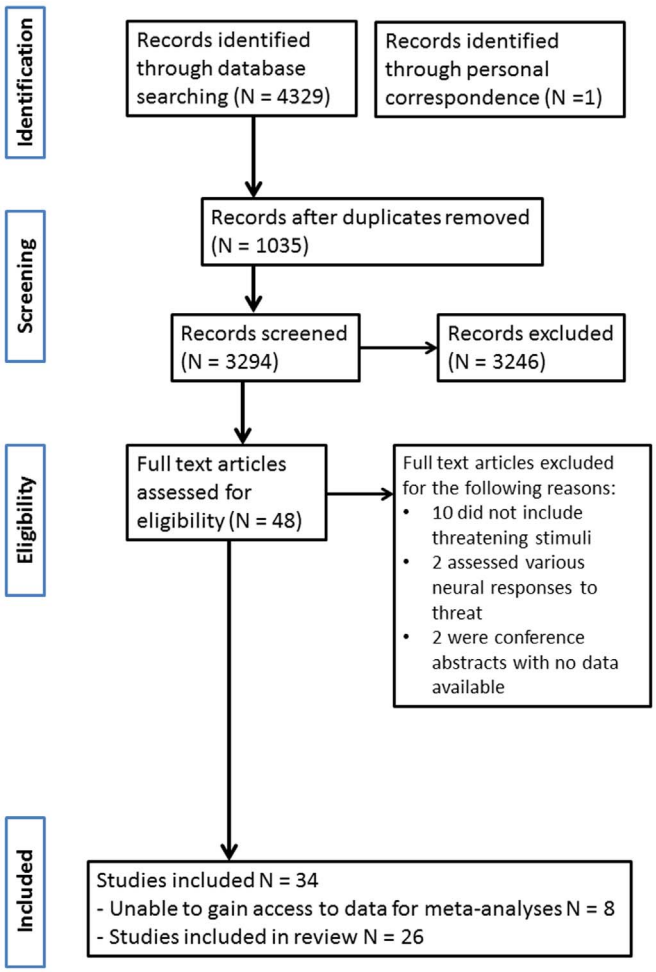

Fig. 1. Literature search flow diagram.

stimulus presentation time in milliseconds (ms), the study design (between subjects, within subjects), and the proportion of female participants in the study, was also extracted from the articles for the purposes of meta-regression.

Where appropriate, information regarding psychopathology was extracted from the studies. This information was extracted from the three studies that investigated the effects of a single dose of intranasal oxytocin on attentional bias to disorder-specific stimuli. This information consisted of self-reported anxiety measured with the Depression, Anxiety and Stress Scale (DASS) and Spielberger State and the Trait Anxiety Inventory (STAI), self-reported depression measured with the DASS and Beck Depression Inventory (BDI), and self-reported ED psychopathology measured with the Eating Disorder Examination Questionnaire. Self-reported anxiety and depression scores were converted to standardised z-scores prior to being included in the meta-regression.

Finally, we also conducted a systematic review of studies investigating the effects of a single dose of oxytocin on behavioural approach and avoidance. We were not able to conduct a meta-analysis due to a small number of studies and differences in outcome measures used.

2.5. Tasks used to examine threat processing

2.5.1. Startle response

Seven studies investigated the effects of a single dose of intranasal oxytocin on the physiological response to general threat and learned threat (Table 1). Four studies used generally threatening stimuli, including angry faces, images of threatening scenes, and predictable and unpredictable electric shocks (Grillon et al., 2013; Leknes et al., 2013; Prehn et al., 2013; Striepens et al., 2012). Three studies investigated the effects of oxytocin on startle response to learned threat cues after conditioning and on learned threat following extinction (Acheson et al., 2013; Eckstein et al., 2015, 2016). The outcomes measures included eye blink magnitude, skin conductance response, pupil dilation, and facial electromyography (EMG). All studies included only healthy individuals. One study reported data separately for male and female participants (Grillon et al., 2013).

2.5.2. Approach and avoidance responses

Five studies investigated the effects of a single dose of intranasal oxytocin on approach and avoidance responses to threatening images including angry faces and general negative stimuli (Table 1). In this experimental paradigm participants are presented with threatening and neutral stimuli one at a time and asked to respond either by pulling or pushing a lever as quickly as possible. Pushing the lever shrinks the stimulus on the screen indicating avoidance of the stimulus, whereas pulling the lever zooms in on the stimulus on the screen indicating approach towards the stimulus. One study reported approach reaction times (Preckel et al., 2014) and another study reported reaction times for both approach and avoidance responses (Radke et al., 2017). Two other studies reported approach-avoidance bias scores (Mitchell et al., 2016; Radke et al., 2013). Positive bias scores indicated approach and negative bias scores indicated avoidance. Finally, one study did not report the relevant data but provided test statistics (Theodoridou et al., 2013). Four of the studies only included healthy individuals, one included people with alcohol dependence disorder, and one explored the effects of oxytocin in low and highly anxious people. Effect sizes and confidence intervals are reported for the four studies that provided the relevant data.

2.5.3. Attentional responses

Two methods were used to investigate the effects of a single dose of intranasal oxytocin on attentional responses to threat: attentional bias towards threatening images relative to neutral images and fixation towards regions of interest within threatening images (Table 1). Seven studies investigated the effects of a single dose of intranasal oxytocin on attentional bias towards social threat, namely images of angry and disgusted faces. Six of the studies examined attentional bias using the dot-probe paradigm and one used a spatial cueing task. In these tasks participants are initially presented with a fixation cross followed by the presentation of a threatening stimulus either alone on one side of a computer screen or in combination with a neutral stimulus on opposite sides of the screen. After the threatening stimulus has disappeared, a target is presented either in the same place where the threatening stimulus was or on the opposite side of the screen. Participants are then asked to identify the target by pressing the appropriate key on a keyboard. In the dot-probe tasks attentional bias scores were calculated from the response times, subtracting response times in trials, where the target replaced a neutral stimulus from response times in trials, where the target replaced the threatening stimulus. Positive scores indicate increased attention towards the threatening stimuli while negative scores indicate reduced attention towards the threatening stimuli.

In the spatial cueing task attentional bias and avoidance were investigated separately by examining reaction times in congruent trials, in which the target was presented in the same side of the screen as the threatening image, and incongruent trials, in which the target was presented on the other side of the screen relative to the threatening image (Ellenbogen et al., 2012). The final attentional bias scores are calculated by subtracting response time in the congruent neutral trials from response times in the congruent threatening trials. The attentional avoidance scores are scores are calculated by subtracting response time in the incongruent neutral trials from response times in the incongruent threatening trials. Thus, as above and despite different methods, positive scores indicate increased attention towards the threatening stimuli

Table 1
Study characteristics.

Study	Design	Dose (IU)	Dose-to-task interval	Group N	Age Mean (SD)	Task	Measure	Stimuli	ES [95% CI]	Power (80%)
Healthy										
Acheson et al. (2013)	Between subjects	24 IU	45 min	Healthy Oxytocin = 22 Placebo = 22% female = 47.75	Oxytocin = 28.2 (6.0) Placebo = 35.5 (21.1)	Fear conditioning (late acquisition)	EMG startle magnitude	Conditioned stimulus	0.03 [−0.57, 0.62]	No
Bertsch et al. (2013)	Between subjects	24 IU	45 min	Healthy Oxytocin = 21 Placebo = 20% female = 100	Oxytocin = 24.6 (3.9) Placebo = 24.4 (4.4)	Fixation during emotion recognition task	Fixation changes	Eyes of angry faces Mouth of angry faces	−0.12 [−0.74, 0.49] 0.31 [−0.31, 0.92]	No
Brune et al. (2013)	Within subjects (sessions 5–7 days apart)	24 IU	45 min	Healthy N = 13% female =76.92	25.7 (6.76)	Dot-probe (200 ms/500 ms stimulus presentation with 500 ms ITI)	Attentional bias	Angry faces	−1.64 [−2.47, −0.81]	No
Clark-Elford et al. (2015)	Within subjects (sessions 7 days apart)	24 IU	45 min	Healthy N = 26% female = 0	26.00 (6.32)	Dot-probe (500 ms stimulus presentation with 500–1250 ms ITI)	Attentional bias	Angry faces	0.04 [−0.35, 0.42]	No
Domes et al. (2013)	Between subjects	24 IU	40 min	Healthy Oxytocin = 30 Placebo = 32% female = 0	Oxytocin = 23.9 (0.4) Placebo = 24.4 (0.5)	Fixation during emotion recognition task	Relative fixation duration	Eyes of angry faces	0.28 [−0.78, 0.22]	No
Eckstein et al. (2015)	Between subjects	24 IU	30 min	Healthy Oxytocin = 18 Placebo = 18% female = 0	Oxytocin = 25.20 (4.46) Placebo = 24.03 (4.08)	Fear conditioning (late acquisition)	Skin conductance startle response	Conditioned stimulus	0.50 [−0.15, 1.16]	No
Eckstein et al. (2016)	Between subjects	24 IU	30 min	Healthy Oxytocin = 17 Placebo = 30% female = 0	Oxytocin = 24.17 (3.58) Placebo = 24.61 (4.28)	Fear conditioning (late acquisition)	Skin conductance startle response	Conditioned stimulus	0.72 [0.10, 1.33]	No
Ellenbogen et al. (2012)	Between subjects	24 IU	45 min	Healthy Oxytocin = 29 Placebo = 28% female = 52.63	Oxytocin = 23.2 (3.3) Placebo = 23.6 (3.5)	SCT: Engagement (masked stimulus presentation with 1850–2500 ms ITI) SCT: Engagement (200 ms stimulus presentation with 1850–2500 ms ITI) SCT: Engagement (750 ms stimulus presentation with 1850–2500 ms ITI) SCT: Avoidance (masked stimulus presentation with 1850–2500 ms ITI) SCT: Avoidance (200 ms stimulus presentation with 1850–2500 ms ITI) SCT: Avoidance (750 ms stimulus presentation with 1850–2500 ms ITI)	Attentional bias	Angry faces	0.10 [−0.42, 0.61] 0.00 [−0.51, 0.51] −0.12 [−0.64, 0.39] 0.09 [−0.42, 0.61] −0.31 [−0.83, 0.21] 0.03 [−0.48, 0.55]	No
Grillon et al. (2013)	Within subjects (N of days between sessions NR)	24 IU	55 min	Healthy Male = 24	NR	Electric shock induced startle during cue presentation task	Eye blink startle response	Predictable shock Unpredictable shock	0.03 [−0.37, 0.43] 0.19 [−0.21, 0.60]	No
				Healthy Female = 19	NR			Predictable shock Unpredictable shock	−0.03 [−0.48, 0.42] 0.07 [−0.38,	

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Table 1 *(continued)*

Study	Design	Dose (IU)	Dose-to-task interval	Group N	Age Mean (SD)	Task	Measure	Stimuli	ES [95% CI]	Power (80%)
									0.52]	
Hubble et al. (2017)	Within subjects (sessions 14 days apart)	24 IU	30 min	Healthy N = 30% female = 0	20.98 (4.55)	Fixation during emotion recognition task	Relative fixation duration	Eyes of angry faces Mouth of angry faces Eyes of disgusted faces Mouth of disgusted faces	−0.11 [−0.47, 0.24] −0.23 [−0.60, 0.13] 0.005 [−0.35, 0.36] 0.005 [−0.35, 0.36]	No
Kim et al. (2014b)	Within subjects (sessions 4–7 days apart)	40 IU	45 min	Healthy N = 33% female = 100	22.18 (2.14)	Dot-probe (500 ms stimulus presentation with 750 ms ITI)	Attentional bias	Angry faces Disgusted faces	−0.21 [−0.55, 0.14] −0.53 [−0.90, −0.17]	No
Kim et al. (2014c)	Within subjects (sessions 7 days apart)	40 IU	45 min	Healthy N = 31% female = 100	22.2 (2.2)	Dot-probe (1000 ms stimulus presentation with 750 ms ITI)	Attentional bias	Angry faces Disgusted faces	−0.20 [−0.55, 0.16] −0.47 [−0.84, −0.10]	No
Leknes et al. (2013)	Within subjects (N of days between sessions NR)	40 IU	40 min	Healthy N = 39% female = 51.28	26 (range = 20–39)	Startle response during emotion evaluation task	Pupil dilation startle response	Angry faces	0.44 [0.11, 0.77]	Yes
Lischke et al. (2012a)	Within subjects (session 4 weeks apart)	24 IU	45 min	Healthy N = 14% female = 100	23.79 (2.32)	Fixation during passive viewing of scenes	Fixation duration Fixation counts	Predefined ROI in negative scenes	0.00 [−0.52, 0.52] 0.37 [−0.17, 0.91]	No
Lischke et al. (2012b)	Between subjects	24 IU	45 min	Healthy Oxytocin = 20 Placebo = 24% female = 0	Total: 26.09 (3.41)	Fixation during emotion recognition	Relative fixation duration	Eyes of angry faces Mouth of angry faces	0.38 [−0.22, 0.97] −0.45 [−1.04, 0.15]	No
Preckel et al. (2014)	Between subjects	24 IU	45 min	Healthy Oxytocin = 33 Placebo = 34% female = 100	Oxytocin = 23.61 (2.71) Placebo = 23.74 (2.47)	Approach / avoidance task	Approach reaction time (ms)	Negative social stimuli Negative non-social stimuli	0.34 [−0.14, 0.83] −0.07 [−0.55, 0.41]	No
Prehn et al. (2013)	Between subjects	24 IU	45 min	Healthy Oxytocin = 23 Placebo = 24% female =	Oxytocin = 25.78 (3.37) Placebo = 26.38 (3.49)	Startle response during emotion recognition task	Pupil dilation startle response	Angry male faces Angry female faces	0.54 [−0.04, 1.12] 0.26 [−0.31, 0.84]	No
Radke et al. (2013)	Within subjects (sessions 14 days apart)	24 IU	45 min	Healthy N = 24% female = 0	21.46 (1.93)	Approach / avoidance task	Approach / avoidance bias	Angry faces	0.52 [0.09, 0.95]	No
Radke et al. (2017)	Between subjects	24 IU	45 min	Healthy Oxytocin = 24 Placebo = 28% female = 0	Total: 22.4 (3)	Approach / avoidance task	Approach reaction time (ms) Avoidance reaction time (ms)	Angry faces	−0.51 [−1.07, 0.04] −0.56 [−1.11, −0.002]	No
Striepens et al. (2012)	Between subjects	24 IU	45 min	Healthy Oxytocin = 36 Placebo = 33% female = 0	Oxytocin = 24.28 (2.86) Placebo = 25.03 (2.56)	Startle response to negative images	Eye blink magnitude startle response	Negative IAPS images	0.70 [0.21, 1.18]	
Theodoridou et al. (2013)	Between subjects	24 IU	35 min	Healthy Oxytocin = 60 Placebo = 60%	Total: 22.4 (range = 18.1–43.8)	Approach / avoidance task	Approach reaction time (ms) Avoidance reaction	Angry faces Disgusted faces Angry faces	NR[a]	No

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Table 1 *(continued)*

Study	Design	Dose (IU)	Dose-to-task interval	Group N	Age Mean (SD)	Task	Measure	Stimuli	ES [95% CI]	Power (80%)
				female = 50			time (ms)	Disgusted faces		
Clinical										
Bertsch et al. (2013)	Between subjects	24 IU	45 min	BPD Oxytocin = 19 Placebo = 19% female = 100	Oxytocin = 23.2 (5.3) Placebo = 24.9 (5.5)	Fixation during emotion recognition task	Fixation changes	Eyes of angry faces Mouth of angry faces	−0.70 [−1.33, −0.06] −0.21 [−0.85, 0.44]	No
Brune et al. (2013)	Within subjects (sessions 5–7 days apart)	24 IU	45 min	BPD N = 13% female = 61.54		Dot-probe (200 ms/500 ms stimulus presentation with 500 ms ITI)	Attentional bias	Angry faces	1.30 [0.56, 2.04]	No
Clark-Elford et al. (2015)	Within subjects (sessions 7 days apart)	24 IU	45 min	Highly anxious people N = 16% female = 0	27.13 (9.25)	Dot-probe (500 ms stimulus presentation with 500–1250 ms ITI)	Attentional bias	Angry faces	−0.42 [−0.93, 0.09]	No
Domes et al. (2016)	Between subjects	24 IU	60 min	Depression Oxytocin = 22 Placebo = 21% female = 58.14	Oxytocin = 46.7 (11.1) Placebo = 47.2 (9.0)	Dot-probe (100 ms stimulus presentation with 750–1500 ms ITI) Dot-probe (600 ms stimulus presentation with 750–1500 ms ITI) Dot-probe (1200 ms stimulus presentation with 750–1500 ms ITI)	Attentional bias	Angry faces	0.50 [−0.10, 1.10] 0.14 [−0.46, 0.73] −0.33 [−0.93, 0.27]	No
Kim et al. (2014b)	Within subjects (sessions 4–7 days apart)	40 IU	45 min	AN N = 31% female = 100	23.10 (9.35)	Dot-probe (500 ms stimulus presentation with 750 ms ITI)	Attentional bias	Angry faces Disgusted faces	0.50 [0.13, 0.87] −0.28 [−0.64, 0.08]	No
Kim et al. (2014a)	Within subjects (sessions 4–7 days apart)	40 IU	45 min	AN N = 31% female = 100	23.10 (9.35)	Dot-probe (1000 ms stimulus presentation with 750 ms ITI)	Attentional bias	Food-related stimuli Body shape-related stimuli Body weight-related stimuli	−0.40 [−0.77, −0.03] −0.38 [−0.75, −0.02] 0.02 [−0.33, 0.37]	No
Kim et al. (In prep)	Within subjects (sessions	40 IU	45 min	BN N = 31% female = 100	23.87(3.99)	Dot-probe (500 ms stimulus presentation with 750 ms ITI) Dot-probe (1000 ms stimulus presentation with 750 ms ITI)	Attentional bias	Angry faces Disgusted faces Food-related stimuli Body shape-related stimuli Body weight-related stimuli Angry faces Disgusted faces Food-related stimuli Body shape-related stimuli Body weight-related stimuli	−0.22 [−0.57, 0.14] 0.09 [−0.26, 0.44] 0.12 [−0.23, 0.47] 0.36 [−0.0002, 0.73] −0.005 [−0.36, 0.35] −0.47 [−0.84, −0.10] 0.04 [−0.31, 0.39] 0.09 [−0.26, 0.44] 0.08 [−0.27, 0.43] 0.28 [−0.08, 0.64]	No

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Table 1 *(continued)*

Study	Design	Dose (IU)	Dose-to-task interval	Group N	Age Mean (SD)	Task	Measure	Stimuli	ES [95% CI]	Power (80%)
Leppanen et al. (2017a)	Within subjects (sessions 1–5 days apart)	40 IU	55 min (before smoothie challenge) 80 min (after smoothie challenge)	AN N = 30% female = 100	26.2 (6.82)	Dot-probe (500 ms stimulus presentation with 500 ms ITI) Dot-probe (500 ms stimulus presentation with 1250 ms ITI) Dot-probe (500 ms stimulus presentation with 500 ms ITI) Dot-probe (500 ms stimulus presentation with 1250 ms ITI)	Attentional bias	Food-related stimuli	0.26 [−0.11, 0.62] −0.22 [−0.58, 0.14] −0.47 [−0.85, −0.10] 0.02 [−0.34, 0.38]	No
Mitchell et al. (2016)	Within subjects (sessions 7 days apart)	40 IU	30 min	Alcohol dependence Disorder N = 32% female = 40.63	28.9 (7.15)	Approach / avoidance task	Approach / avoidance bias	Negative IAPS images	0.16 [−0.19, 0.51]	No

ES = effect size estimate (either standardised mean difference or standardised mean change); IU = international unit; SD = standard deviation; EMG = electromyography; min = minutes; ms = milliseconds; ES = effect size; SCT = spatial cueing task; ITI = inter-trial interval; ROI = region of interest; IAPS = International Affective Picture System; BPD = borderline personality disorder; AN = anorexia nervosa; BN = bulimia nervosa; NR = not reported. The Power column indicates whether or not the studies met our sample size requirement to have 80% power to detect at least a moderate difference between oxytocin and placebo.

[a] Relevant data was not reported in the paper.

and negative scores indicate reduced attention towards the threatening stimuli in both tasks. Further, two of the studies varied the cue presentation duration or used masked cue presentation (Brune et al., 2013; Domes et al., 2016; Ellenbogen et al., 2012).

Three studies investigated attentional bias towards disorder-specific threatening stimuli in ED, namely AN and BN, using the dot-probe paradigm (Table 1). These studies used food, body shape, and body weight related images as threatening stimuli. As above, positive attentional bias scores indicated greater attention towards the disorder-specific threatening images, and negative scores indicated attention away from the stimuli. One of the studies varied the cue presentation duration (Kim et al., In prep), and another study investigated attentional bias with varied inter-trial interval (500 ms or 1250 ms) before and after a smoothie challenge (Leppanen et al., 2017a).

Five studies investigated the effects of a single dose of intranasal oxytocin on attention towards regions of interest (ROIs) in threatening images using eye tracking (Table 1). Three of the studies defined the ROIs as the eye and mouth region of angry and disgusted faces (Bertsch et al., 2013; Domes et al., 2013; Hubble et al., 2017; Lischke et al., 2012b). One study investigated fixation towards predefined regions of interest in threatening scenes taken from the international picture affective system (Lischke et al., 2012a). The ROIs were predefined based on pilot data. The outcome measure was preferential fixation towards the ROIs relative to the rest of the face or the background of the image.

2.6. Statistical analysis

Statistical analyses were conducted with the Metafor package in R (R Core Team, 2015; Viechtbauer, 2010). For studies using between subjects design, standardised, unbiased effect sizes were estimated by calculating Hedges' g (Hedges, 1981) and 95% confidence intervals. For studies using within subjects design standardised mean change with change score standardisation (SMCC) along with 95% confidence intervals was calculated. Where correlation between the two conditions was not reported to calculate the SMCC effect size estimate, the correlation was estimated using the following formula: $\frac{SD1^2 + SD2^2 - SDchange^2}{2 * SD1 * SD2}$ (Morris and DeShon, 2002). The Hedges' g and SMCC effect size estimates are both on the same scale and were interpreted as small (≥ 0.20), medium (≥ 0.50), and large (≥ 0.80). Positive effect sizes indicated oxytocin-induced increase in startle response, approach, and attention towards threatening stimuli. Negative effect sizes indicated oxytocin-induced reduction in startle response, approach, and attention towards threatening stimuli.

Because a number of studies used a variety of paradigms to investigate different aspects of threat processing, the same participants were included in a meta-analysis more than once. To account for confounding effects arising from this, we conducted multivariate meta-analyses with an auto-regressive structure. Between-study heterogeneity was examined with the *Cochran's Q* index. Significant heterogeneity between studies was further investigated with meta-regressions to examine the moderating effects of the following variables: age, the study design, the dose administered (in IU), the type of task used, diagnosis, the type of stimuli used, and the proportion of female participants in the sample. Significance level was set at $p < 0.05$.

Presence of outliers was investigated by calculating standardised residuals of each study included in the meta-analyses. If the Z scores of the standardised residuals exceeded ± 1.96, the study was deemed an influential outlier and was thus removed from the analyses (Viechtbauer and Cheung, 2010).

Publication bias based on funnel plot asymmetry was investigated with Begg's rank correlation test (Begg and Mazumdar, 1994). Additionally, where significant effects were present their robustness was assessed with the Rosenthal's fail-safe N analysis (Rosenthal, 1979). The analysis provides a fail-safe number of studies with non-significant results that would need to be included for the effects to be reduced to

null. The effect is considered robust if the fail-safe N exceeds the Rosenthal criterion, 5k + 10, where k is the number of studies included in the meta-analysis (Rosenthal, 1991).

Finally, we explored whether the included studies had large enough sample sizes to have adequate power to detect an effect of oxytocin on threat processing. According to the power analysis conducted with G*Power (Faul et al., 2007), between subjects studies should have at least 64 participants in each group and within subjects studies should have at least 34 participants, to have 80% power to detect a moderate effect.

3. Results

3.1. Study characteristics

Characteristics of the 26 included studies are presented in Table 1. The effect size (ES) column shows the standardised effect size estimate along with 95% confidence intervals for each study. The power column show whether the study had a sufficiently large sample size to have 80% power to detect at least a moderate (ES ≥ 0.5) effect of oxytocin. Only one study using within subjects design met the sample size requirement of sufficient power. Six other studies using within subjects design came close to having sufficient power (sample size ≥ 30).

3.2. Effects of oxytocin on startle response to threat

The studies investigating the effects of a single dose of intranasal oxytocin on physiological startle response to general threat and learned threat are presented in Fig. 2. Overall, the meta-analysis revealed that intranasal oxytocin significantly increased startle response to threat with a small effect size (ES = 0.32, 95% CI [0.13, 0.52], Z = 3.27, p = 0.001). We further explored the effects of oxytocin on startle response to general threat and learned threat following conditioning. Intranasal oxytocin significantly increased startle response to general threat with a small effect size (ES = 0.30, 95% CI [0.07, 0.53], Z = 2.55, p = 0.011). Oxytocin-induced increase in startle response following conditioning also approached significance with a small effect size (ES = 0.41, 95% CI [−0.01, 0.82], Z = 1.90, p = 0.058).

There was no significant between-study heterogeneity (Q = 11.32, p = 0.297, I^2 = 29.00), and thus, no meta-regressions were conducted. Begg's rank correlation test based on the funnel plot did not indicate there was evidence of significant publication bias (τ = 0.24, p = 0.359, Supplementary Fig. 1). The Rosenthal's fail-safe N analysis revealed that 55 studies with non-significant results would need to be added to the present meta-analysis to reduce the observed effect to null. This exceeds the Rosenthal criterion (55 > (5 × 7 + 10) = 45) suggesting that the effect observed was robust.

3.3. Effects of oxytocin on attentional responses towards threat

3.3.1. Attentional bias towards social threat

The studies investigating the effects of a single dose of intranasal oxytocin on attentional bias towards social threat are presented in Fig. 3. Overall, a single dose of intranasal oxytocin did not significantly influence attentional bias towards social threat (ES = −0.10, 95% CI [−0.50, 0.29], Z = −0.51, p = 0.612). We then further explored the effects within the healthy and clinical populations. Among the healthy individuals, oxytocin did not significantly impact attentional bias towards social threat (ES = −0.42, 95% CI [−1.07, 0.23], Z = −1.26, p = 0.208). There was also no significant effects of oxytocin among the mixed clinical group (ES = 0.13, 95% CI [−0.35, 0.61], Z = 0.54 p = 0.593).

The overall meta-analysis revealed significant between-study heterogeneity (Q = 73.53, p < 0.001), which was explored further with meta-regressions. Together the stimuli used, the stimulus presentation time, and the diagnostic group significantly moderated some of the between-study heterogeneity (QM = 43.41, p < 0.0001), still leaving significant residual heterogeneity (QR = 24.60, p = 0.004). This suggests that the effects of oxytocin on attentional bias were the strongest among highly anxious people and people with BPD when the angry faces were presented for 500 ms or less time. However, only one study included people with BPD and only one study included highly anxious people and, thus, these findings should be interpreted with caution. The proportion of female participants in the study (QM = 0.03, p = 0.859), the study design (QM = 0.11, p = 0.744), age (QM = 0.295, p = 0.589), the task used (QM = 0.29, p = 0.963), and the dose administered (QM = 0.01, p = 0.929) did not significantly moderate the between study heterogeneity.

The Begg's rank correlation test based on the funnel plot asymmetry did not reveal evidence of significant publication bias (τ = 0.09, p = 0.565; Supplementary Fig. 2).

3.3.2. Attentional bias towards disorder-specific threat

The studies investigating the effects of a single dose of intranasal oxytocin on attentional bias towards disorder-specific threatening stimuli are presented in Fig. 4. Only studies including people with ED, namely AN and BN, had investigated these effects. The disorder-specific threatening stimuli consisted of negative body shape related, body weight related, and food related images.

The meta-analysis showed that overall a single dose of intranasal

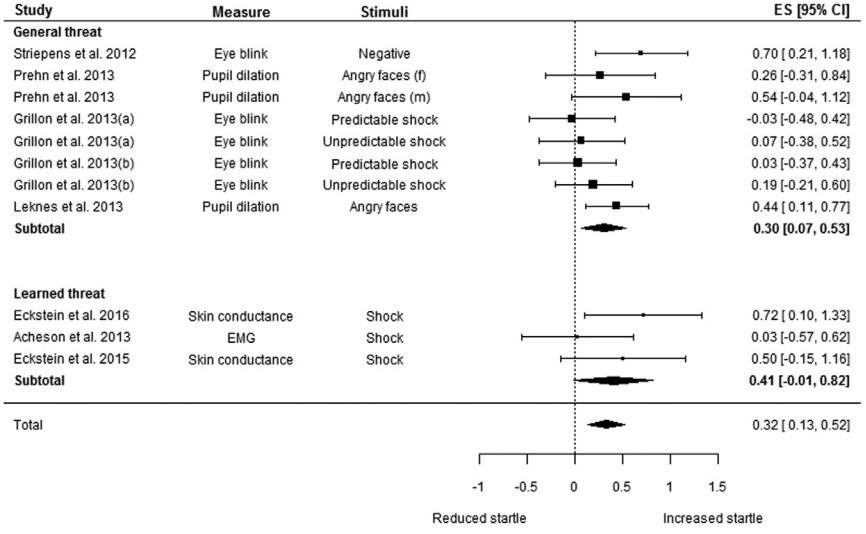

Fig. 2. Forest plot of studies investigating the effects of intranasal oxytocin on the startle response to threat. Grillon et al. (2013)(a) = female participants, Grillon et al. (2013)(b) = male participants, Angry faces (f) = angry female faces, Angry faces (m) = angry male faces, EMG = electromyography.

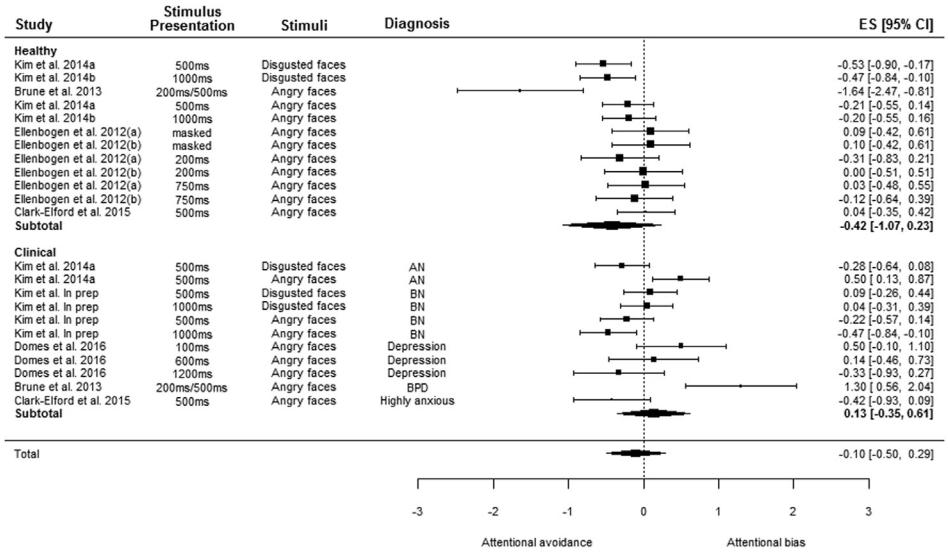

Fig. 3. Forest plot of studies investigating the effects of intranasal oxytocin on the attentional bias towards social threat. Ellenbogen et al. (2012) (a) = congruent trials, Ellenbogen et al. (2012) (b) = incongruent trials, AN = anorexia nervosa; BN = bulimia nervosa; BPD = borderline personality disorder, ms = milliseconds.

oxytocin did not significantly influence attentional bias towards disorder-specific stimuli in eating disorders (ES = −0.05, 95% CI [−0.28, 0.18], Z = −0.43, p = 0.670). We further explored the effect of a single dose of oxytocin separately on the different types of disorder specific stimuli used. Intranasal oxytocin did not significantly influence attentional bias towards negative body shape related stimuli (ES = −0.07, 95% CI [−0.66, 0.52], Z = −0.23, p = 0.818), body weight related stimuli (g = 0.09, 95% CI [−0.12, 0.30], Z = 0.86, p = 0.388), or food related stimuli (ES = −0.10, 95% CI [−0.36, 0.15], Z = −0.79, p = 0.429).

There was significant between-study heterogeneity (Q = 24.52, p = 0.017), which was explored further with meta-regressions. Diagnostic group significantly explained the between-study heterogeneity (QM = 6.37, p = 0.012), still leaving some unaccounted heterogeneity that approached significance (QR = 18.15, p = 0.078). This suggests that people with BN showed greater oxytocin induced increase in attentional bias towards disorder-specific stimuli (ES = 0.15, 95% CI [−0.03, 0.33], Z = 1.66, p = 0.096), whereas people with AN showed greater oxytocin-induced increase in attentional avoidance of disorder-specific stimuli (ES = −0.16, 95% CI [−0.33, 0.03], Z = −1.92, p = 0.055). However, neither of these effects reached significance and only one study investigated the effects of oxytocin in people with BN. Therefore, these findings should be interpreted with caution. Self-reported anxiety (QM = 0.20, p = 0.655), self-reported depression (QM = 1.41, p = 0.235), self-reported eating disorder symptomatology (QM = 0.04, p = 0.846), the stimuli used (QM = 1.56, p = 0.458), age (QM = 0.01, p = 0.937), and variations in the task used (QM = 0.08, p = 0.995) did not significantly moderate the between study heterogeneity. The proportion of female participants in the study, the dose administered, and the study design were not entered as predictors in the meta-regression as all included studies recruited only female participants, used the same within subjects design, and administered the same dose.

The Begg's rank correlation test based on the funnel plot asymmetry did not reveal evidence of significant publication bias (τ = −0.15, p = 0.510; Supplementary Fig. 3).

3.3.3. Fixation towards regions of interest

The studies investigating the effects of a single dose of intranasal oxytocin on fixation towards ROIs in threatening images are presented in Fig. 5. The meta-analysis showed that a single dose of oxytocin did not significantly influence fixation towards ROIs in threatening images (g = −0.08, 95% CI [−0.23, 0.08], Z = −0.96, p = 0.339).

There was significant between-study heterogeneity (Q = 22.65, p = 0.031), which was explored further with meta-regressions. The ROI

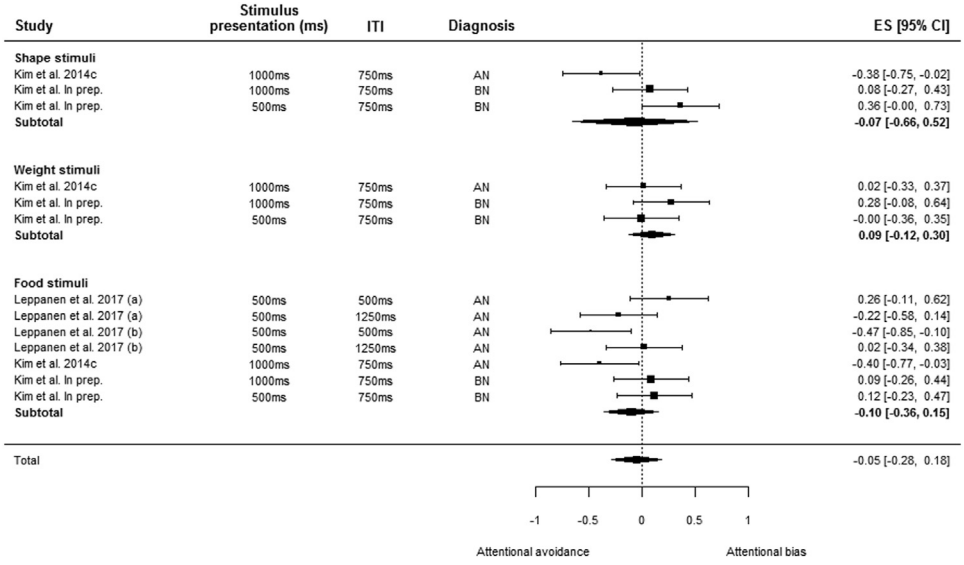

Fig. 4. Forest plot of studies investigating the effects of intranasal oxytocin on attentional bias towards disorder specific threat. Leppanen et al. (2017a) (a) = before smoothie challenge, Leppanen et al. (2017a) (b) = after smoothie challenge, AN = anorexia nervosa, BN = bulimia nervosa, ms = milliseconds, ITI = inter-trial interval.

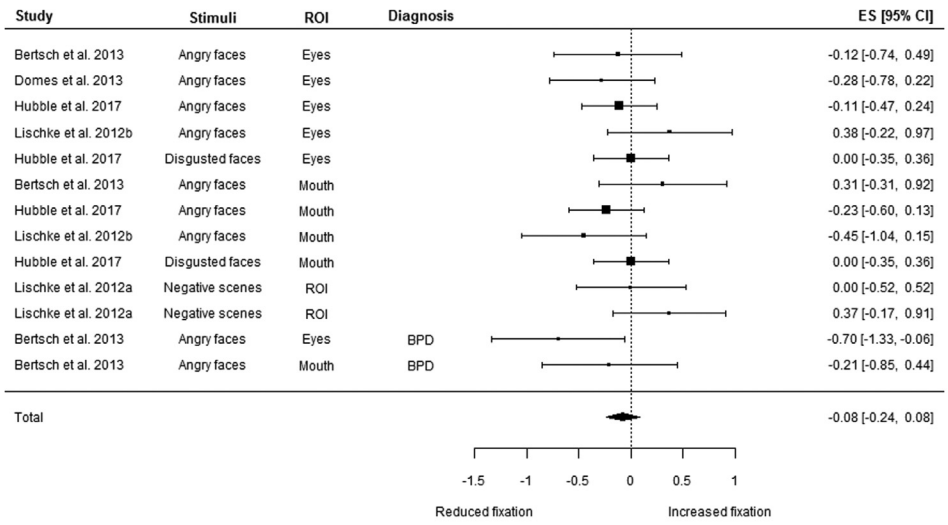

Fig. 5. Forest plot of studies investigating the effects of intranasal oxytocin on fixation towards regions of interest in threating images. ROI = region of interest, BPD = borderline personality disorder. Lischke et al. (2012a) used predefined regions of interest based on pilot data.

used (QM = 1.53, p = 0.465), age (QM = 0.01, = 0.931), the proportion of female participants in the study (QM = 0.28, p = 0.597), the study design (QM = 0.65, p = 0.419), the task used (QM = 1.53, p = 0.216), how fixation was measured (QM = 3.02, p = 0.388), the type of stimuli used (QM = 2.87, p = 0.238), the diagnosis (QM = 1.80, p = 0.179), and the dose administered (QM = 0.25, p = 0.615) did not significantly moderate the heterogeneity.

Begg's rank correlation test based on the funnel plots did not reveal any significant publication bias ($\tau = -0.17$, p = 0.435, Supplementary Fig. 4).

3.4. Effects of oxytocin on approach and avoidance responses to threat

It was not possible to conduct a full meta-analysis of the studies investigating the effects of oxytocin on approach and avoidance responses due to the small number of studies and the variety of different outcome measures used. The significance of the findings from each study was then investigated by examining the 95% confidence intervals of the effect size estimates according to the guidelines stated in the Cochrane Handbook for Systematic Reviews (Higgins and Green, 2011).

Two studies used a between subjects design to investigate the effects of a single dose of intranasal oxytocin on approach and avoidance reaction times (Preckel et al., 2014; Radke et al., 2017). The study by Preckel et al. (2014) examined only approach reaction times to social and non-social threat. The study found that intranasal oxytocin led to a small reduction in reaction times when approaching threatening social stimuli (ES = 0.34, 95% CI [−0.14, 0.83], total N = 67). There was a negligible reduction in reaction times when approaching non-social threatening stimuli (ES = 0.07, 95% CI [−0.41, 0.55], total N = 67). However, in both cases the 95% confidence intervals overlapped zero indicating that neither effect was statistically significant. Conversely, Radke et al. (2017) found that oxytocin led to a non-significant increase in approach reaction times towards social threat with a moderate effect size (ES = −0.51, 95% [−1.07, 0.04], total N = 52), and a significant increase in avoidance reaction times with a moderate effect size (ES = −0.56, 95% CI [−1.11, −0.002], total N = 52). These contradictory findings may be some way explained by the differences in sample size.

Two studies by Radke et al. (2013) and Mitchell et al. (2016) used within subjects design to investigate approach/avoidance bias in healthy people and people with alcohol dependence disorder respectively. Radke et al. (2013) found a significant oxytocin-induced increase in approach bias towards threatening social stimuli with a moderate effect size among healthy individuals (ES = 0.52, 95% CI [0.09, 0.95], N = 24). Similarly, Mitchell et al. (2016) found a negligible oxytocin-induced increase in approach bias towards threatening non-social stimuli in people with alcohol dependence disorder (ES = 0.16, 95% CI [−0.19, 0.51], N = 32), although this did not reach significance. Taken together these findings suggest that oxytocin may increase approach bias towards threatening stimuli, particularly threatening social stimuli. However, further research is needed to confirm this finding.

Finally, the study by Theodoridou et al. (2013) used a between subjects design to investigate the effects of intranasal oxytocin on both approach and avoidance responses to social threat (total N = 120). The authors did not report the relevant data to allow effect sizes to be calculated, but reported test statistics that showed no significant effect of intranasal oxytocin on approach or avoidance responses (Theodoridou et al., 2013).

Taken together these studies indicate that there is uncertainty about the effects of intranasal oxytocin on approach and avoidance responses to threatening stimuli. Indeed, the 95% confidence interval ranges in all studies were very wide (Higgins and Green, 2011). Still, there was some indication that oxytocin may influence approach responses to threatening stimuli, particularly social threat. However, it is of interest that the two studies that showed significant effect size estimates also had smaller sample sizes (within subjects N = 24; between subjects N = 52) than the other studies reporting no significant effects of oxytocin (within subjects N = 32, between subjects N = 67 − 120). Thus, further research with larger samples is needed.

4. Discussion

The aim of the present review was to examine the effects of a single dose of intranasal oxytocin on threat processing in healthy and clinical populations. The majority of the studies included only healthy individuals and no studies investigated the influence of intranasal oxytocin on the physiological startle response among clinical populations. The findings revealed that intranasal oxytocin significantly increased the startle response to threatening stimuli in healthy individuals with a small effect size. However, a single dose of intranasal oxytocin did not significantly influence attentional bias towards social threat or disorder specific threat, or fixation to regions of interest in threatening stimuli. The findings for effects of a single dose of oxytocin on approach and avoidance responses towards threat were inconsistent, calling for further research with large samples.

In preclinical studies, oxytocin has been shown to facilitate extinction and generally inhibit anxiety- and fear-related responses and behaviours in rodents (Calcagnoli et al., 2015; Huber et al., 2005; Neumann and Slattery, 2016; Viviani et al., 2011). Conversely, the present review did not find evidence of significant overall effects of intranasal oxytocin on attentional or behavioural approach or

avoidance responses to threat in humans. Instead, we found that a single dose of intranasal oxytocin significantly increased startle response to threatening stimuli in healthy individuals. Based on the present findings, in healthy humans, oxytocin appears to primarily influence the salience of threatening stimuli, possibly indicating that oxytocin plays a role in arousal but not behavioural responding in humans. This interpretation is supported by findings from recent neuroimaging studies investigating neural responses to social threat in healthy humans. These studies have reported oxytocin-induced increases in neural activation in regions, including the amygdala, which are associated with processing of salience and arousal (Frijling et al., 2016; Gorka et al., 2015; Koch et al., 2016). This interpretation is also supported by findings from a previous meta-analysis that found that a single dose of intranasal oxytocin improved early detection of anger and late detection of fear in healthy humans (Shahrestani et al., 2013). Furthermore, a recent meta-analytic review by our group found that a single dose of oxytocin improved recognition of basic emotions, primarily fear and disgust, among healthy individuals (Leppanen et al., 2017b). Thus, these findings suggest that rather than having global effects on threat processing, oxytocin may influence specific aspects of threat processing, such as arousal.

Regarding clinical populations, the present review found that a single dose of intranasal oxytocin did not significantly influence attentional bias towards social threat among clinical populations. These findings are somewhat surprising considering the number of systematic reviews suggesting that oxytocin may be an effective new target for treatments in psychiatric disorders alleviating psychopathology in a number of disorders (Bakermans-Kranenburg and van Ijzendoorn, 2013; MacDonald and Feifel, 2013; MacDonald and MacDonald, 2010; Meyer-Lindenberg et al., 2011). One possible interpretation of the present findings is that although it may be effective in targeting certain symptoms, intranasal oxytocin may have little effect on threat processing among the clinical populations. This interpretation is in line with findings from recent work investigating the effectiveness of intranasal oxytocin as a treatment enhancer in depression and BPD, which found that intranasal oxytocin did not improve anxiety or affiliative behaviour during therapy or clinical interview (Brune et al., 2015; MacDonald et al., 2013). Additionally, another study found that intranasal oxytocin did not improve self-reported anxiety among people with social anxiety disorder following exposure therapy (Guastella et al., 2009).

It is of note, however, that the present review was only able to investigate the effects of a single dose of intranasal oxytocin on attentional responses to threat among clinical populations due to lack of studies. Thus, it is possible that intranasal oxytocin does not have a significant effect on threat related attentional or behavioural responses, but may modulate other aspects of threat processing among clinical population. Indeed, a previous meta-analysis found that a single dose of oxytocin reduced the cortisol response to stressful stimuli among clinical populations (Cardoso et al., 2014). Similarly, a recent systematic review reported that intranasal oxytocin may be effective in combating anxiety and stress in disorder characterised by dysfunction of the central nervous system (Chapman et al., 2013). Furthermore, a recent experimental study found that a single dose of oxytocin increased heart rate response to trauma related stimuli while reducing self-reported trauma-related imagery among people with PTSD (Sack et al., 2017). These findings suggest that oxytocin may increase arousal while lowering stress and anxiety responses to threatening stimuli among clinical populations. However, further research with larger samples is still needed.

Another possible interpretation of the present findings is that there is substantial heterogeneity within and between clinical groups that could dilute the true effects of intranasal oxytocin on social threat processing among clinical populations (Lamers et al., 2010; Melartin et al., 2002; Wessman et al., 2009). Indeed, in the present study there was significant between study heterogeneity in the meta-analysis investigating effects of oxytocin on attentional bias towards threat among clinical populations. Findings from preclinical and human studies also suggest that the effects of oxytocin may be mediated by baseline levels of anxiety and other individual differences, such as attachment difficulties (Bartz et al., 2011; Bosch et al., 2005; Olff et al., 2013; Zik and Roberts, 2015). Environmental differences, such as the conditions under which participants are tested, have also been suggested to influence the effects of oxytocin (Bartz et al., 2011; Olff et al., 2013). This suggestion is in line with findings from our previous single dose study, which found that the effects of intranasal oxytocin on attentional bias towards food images in people with AN was dependent not only on whether the task was delivered before or after a smoothie challenge, but also on the inter-trial interval used in the task (Leppanen et al., 2017a). Although, further research could help gain better understanding of the effects of intranasal oxytocin on threat processing among clinical populations, these findings raise some questions about the therapeutic potential and effectiveness of intranasal oxytocin in the treatment of psychiatric disorders.

4.1. Limitations and future directions

The main limitation of this review is the small number of studies investigating the effects of intranasal oxytocin on different aspects of threat processing. Indeed, there were so few studies investigating the effects of a single dose of intranasal oxytocin on behavioural approach and avoidance responses to threatening stimuli, we were not able to conduct a full meta-analysis. Furthermore, only one of the studies reviewed here met our sample size requirement for adequate statistical power. These limitations caused particular concern when reviewing studies investigating the effects of a single dose of oxytocin on approach and avoidance responses. The only two that showed significant effects of oxytocin had smaller sample size than the other similar studies that did not show significant effects of oxytocin. It is possible that these findings were driven by the fact that smaller studies can sometimes overestimate effect sizes even when no true effect is present (Walum et al., 2016; Zhang et al., 2013). Together, these findings suggest that this is still a relatively unexplored field, and as further research with larger samples is needed.

Additionally, there is relative paucity of research investigating the effects a single dose of intranasal oxytocin on threat processing among clinical populations. In the present review we were only able to investigate the effects of a single dose of oxytocin on attentional bias towards threat among clinical populations, but not the physiological startle response, approach and avoidance responses, or fixation response due to a lack of studies. Further research is needed to shed light on the effects of oxytocin on elevated threat sensitivity in psychiatric disorders.

The dot-probe and spatial cueing tasks that were used to examine the effects of oxytocin on attention to social threat demonstrated that variation of task parameters, such as stimulus presentation time and inter-trial interval, had an impact on the effects of oxytocin. These factors added heterogeneity into the meta-analyses and influenced the interpretation of the findings. Short stimulus presentation duration is believed to tap into early attentional responses while longer stimulus duration is believed to examine late attention (Bantin et al., 2016). However, there are still some uncertainties regarding what should be used as the cut-off for early and late attention, with some eye tracking and electroencephalogram (EEG) studies reporting attentional shifts as early as 200 ms after stimulus onset (Bantin et al., 2016; Belyusar et al., 2013; Hedge and Leonards, 2013). Additionally, working memory and executive functioning studies have demonstrated that varying the inter-trial interval can impact performance, with shorter inter-trial intervals utilising more automated responding, while longer inter-trial intervals allow for more controlled responding (Cermak, 1970; Shipstead and Engle, 2013). Thus, to avoid these issues, future studies may benefit from utilising other methods to assess attentional bias, such as eye tracking or EEG, and working towards developing standardised

measures.

Finally, there was significant between study heterogeneity in the meta-analysis investigating the effects of a single dose of oxytocin on attentional bias towards social threat and disorder-specific threat. Despite conducting meta-regressions to investigate moderating effects of age, dose, the type of task used, the type of stimuli used, and study design, we were not able to ascertain the source of the heterogeneity. Factors such as anxiety, attachment style, and alexithymia have been found to influence the effects of oxytocin on a number of different tasks (Bartz et al., 2011; Bosch et al., 2005; Olff et al., 2013; Zik and Roberts, 2015). However, since these variables were not explored or reported in the majority of the included studies we were unable to explore their moderator effects in most of the meta-analyses. Thus, further work investigating potential moderators of the effects of oxytocin on attentional bias towards threatening stimuli would be of interest.

5. Conclusions

The aim of the present review was to examine the effects of a single dose of intranasal oxytocin on threat processing in healthy and clinical populations. The findings revealed that oxytocin significantly increased the physiological startle response to threat in healthy people with a small effect size. Oxytocin did not have significant effects on attentional bias towards social or disorder-specific threat or on fixation towards threatening stimuli among healthy or clinical populations. There was also no convincing evidence that oxytocin significantly influences threat related behavioural approach or avoidance responses. These findings suggest that oxytocin may primarily influence the salience of threatening stimuli possibly indicating that oxytocin plays a role in emotional, but not behavioural processing in humans. Large scale research investigating the effects of oxytocin on physiological startle response among clinical populations would be of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jad.2017.08.041.

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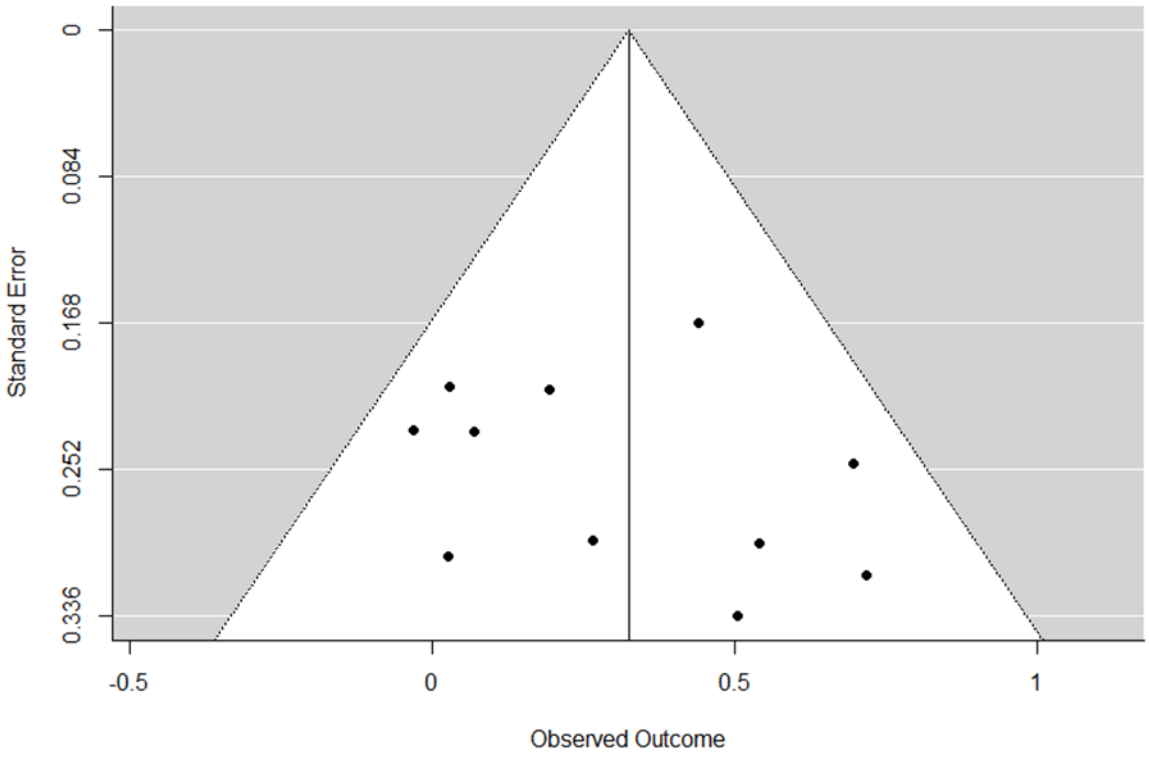
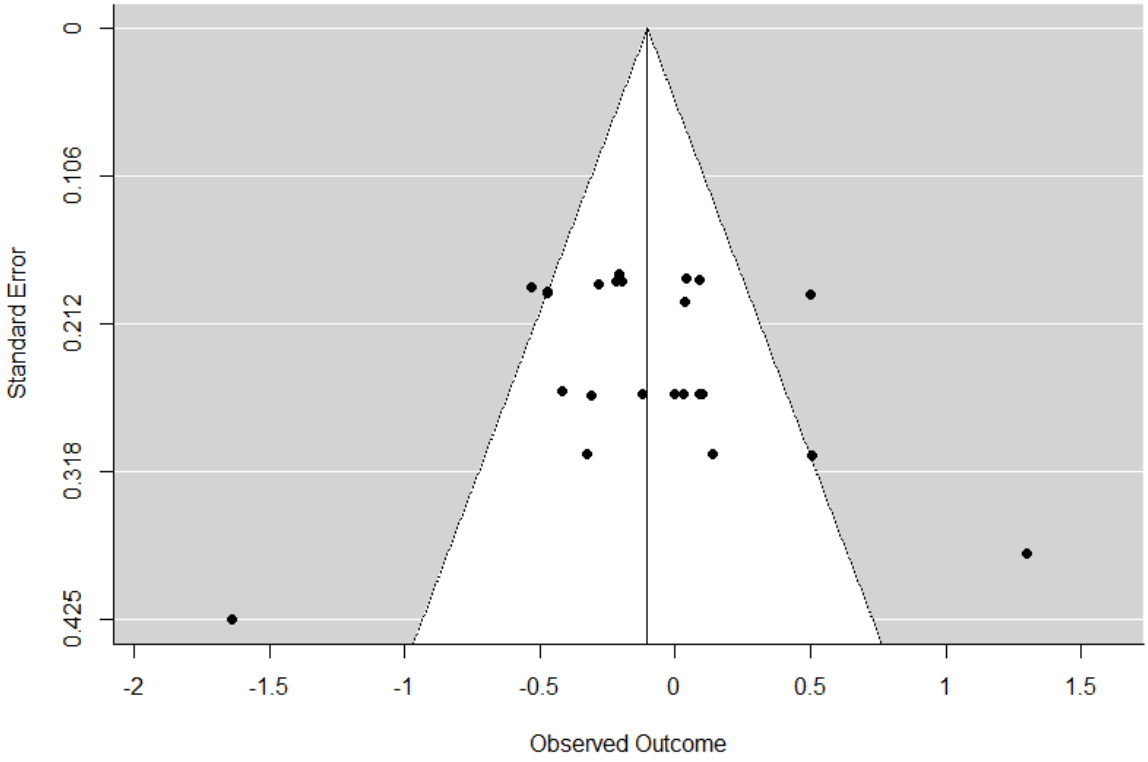
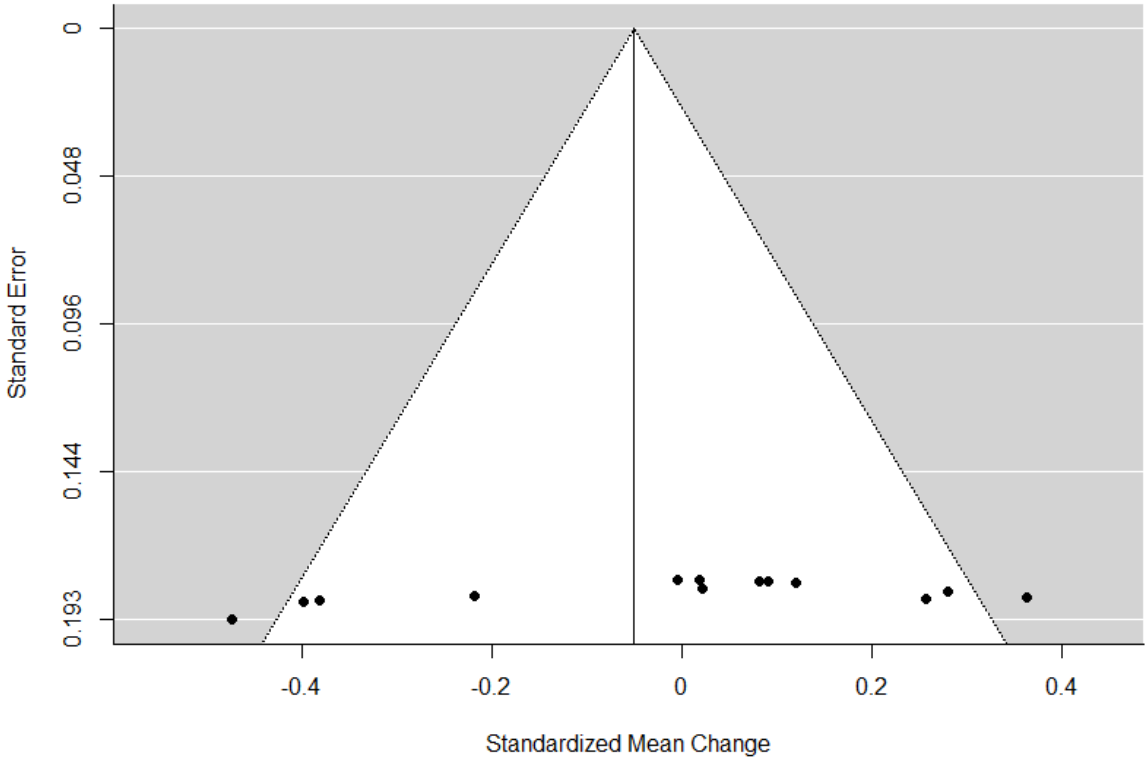
Supplementary Figure 1. Funnel plot of studies investigating the effects of intranasal oxytocin on the startle response to threat



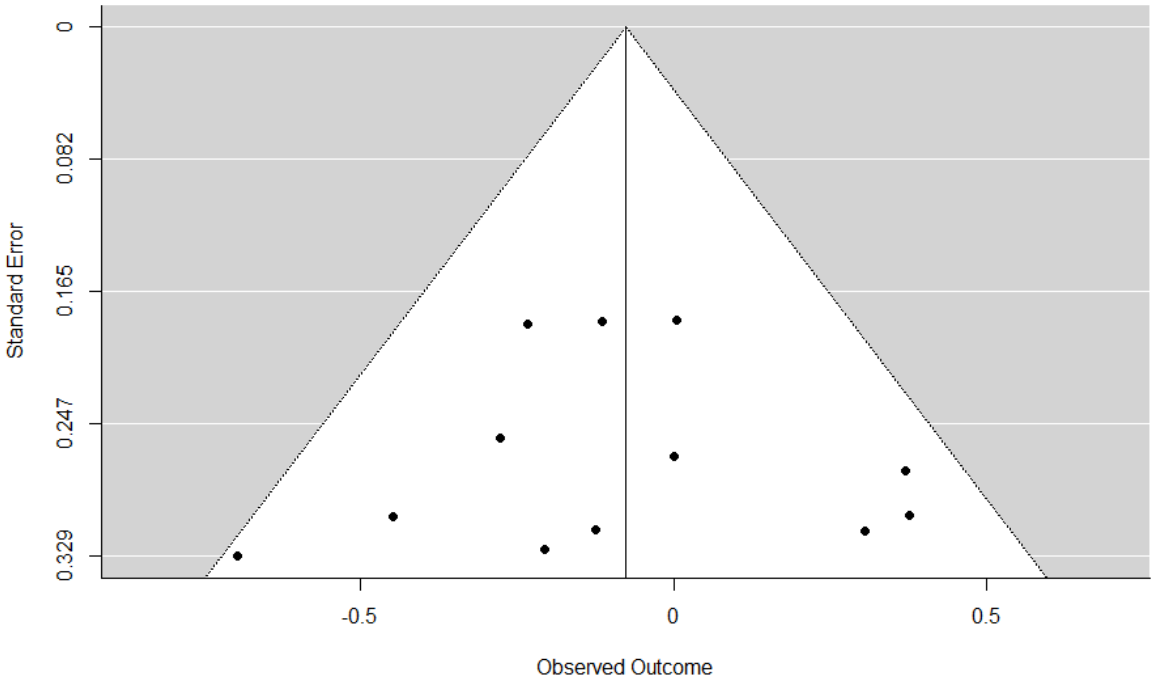
Supplementary Figure 2. Funnel plot of studies investigating the effects of intranasal oxytocin on the attentional bias towards social threat



Supplementary Figure 3. Funnel plot of studies investigating the effects of intranasal oxytocin on attentional bias towards disorder specific threat



Supplementary Figure 4. Funnel plot of studies investigating the effects of intranasal oxytocin on fixation towards regions of interest in threating images



CHAPTER 8:

Meta-analysis of the effects of intranasal oxytocin on interpretation and expression of emotions

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Review article

Meta-analysis of the effects of intranasal oxytocin on interpretation and expression of emotions

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ABSTRACT

Accurate interpretation and appropriate expression of emotions are key aspects of social-cognition. Several mental disorders are characterised by transdiagnostic difficulties in these areas and, recently, there has been increasing interest in exploring the effects of oxytocin on social-emotional functioning.

This review consists of 33 studies. Fifteen of the studies included people with autism spectrum disorder, schizophrenia, borderline personality disorder, frontotemporal dementia, anorexia nervosa, bulimia nervosa, post-traumatic stress disorder, depression, and opioid and alcohol dependence. We conducted ten meta-analyses examining the effects of intranasal oxytocin on expression of emotions, emotional theory of mind, sensitivity to recognise basic emotions, and recognition of basic emotions.

A single dose of intranasal oxytocin significantly improved the recognition of basic emotions, particularly fear, and increased the expression of positive emotions among the healthy individuals. Oxytocin did not significantly influence theory of mind or the expression of negative emotions among the healthy individuals. Finally, intranasal oxytocin did not significantly influence interpretation or expression of emotions among the clinical populations.

1. Introduction

Accurate interpretation of other's emotions, appropriate expression of one's own emotions, and reciprocity within interactions are key aspects of social cognition. In social interaction, emotion expression is believed to be dependent on accurate interpretation of social signals (Hess and Fischer, 2013; Künecke et al., 2014). According to the embodied simulation theory, emotion expression and mimicry, in turn, play an important role in facilitating the interpretation of others' expressions, empathy, and prosocial behaviour in recipients (Gallese, 2005). Indeed, behavioural studies have documented that automatic mimicry of emotions facilitates recognition, whereas blocking mimicry impairs recognition accuracy and sensitivity (Argaud et al., 2016; Duffy and Chartrand, 2015; Künecke et al., 2014; Rychlowska et al., 2014; Schneider et al., 2013). Anomalies in emotion expression also have social and affective consequences, with incongruent emotion expression increasing the desire for greater social distance and negative social evaluation by the recipient (Brown et al., 2015; Szczurek et al., 2012). Similarly, expressive suppression has been found to increase the suppressors' blood pressure, subjective anxiety, and social isolation (Butler et al., 2003; Gross, 2002).

Anomalies in social-emotional functioning are important transdiagnostic features in several psychiatric disorders (Bora and Berk, 2016; Bora and Köse, 2016; Chung et al., 2014; Davies et al., 2016; Henry et al., 2014; Kring and Moran, 2008). Meta-analyses have found that people with eating disorders (EDs), depression, schizophrenia, and autism spectrum disorders (ASD) have similar difficulties in accurate interpretation of emotions, including recognition of basic emotions in faces and tone of voice with small effect sizes and in emotional theory of mind with medium to large effect sizes (Bora and Berk, 2016; Bora and Köse, 2016; Caglar-Nazali et al., 2014; Chung et al., 2014; Uljarevic and Hamilton, 2013). Recent systematic reviews have also found that people with schizophrenia, EDs, depression, ASD, and borderline personality disorder (BPD) display less positive facial affect in response to positive emotional stimuli (Davies et al., 2016; Kring and Moran, 2008). Furthermore, a meta-analysis of 537 task-based fMRI studies in depression, bipolar disorder, schizophrenia, and obsessive-compulsive disorder failed to find significant differences between the disorders in whole brain neural response to social and cognitive tasks (Sprooten et al., 2016). Together these findings suggest that anomalies in social-

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emotional processing in psychiatric disorders may have shared underlying mechanisms. Given social and affective consequences of these difficulties, better understanding of the underlying processes is of interest. One such possible mechanism is the oxytocin system.

Preclinical studies have found that the neuropeptide, oxytocin, may regulate social-emotional functioning (Dölen et al., 2013; Hicks et al., 2012; Lim and Young, 2006; Lukas et al., 2011; Onaka et al., 2012). Endogenous oxytocin has been found to play an important role in the central and medial amygdala, facilitating formation of social bonds, maternal behaviour, and social recognition in rodents (Lim and Young, 2006; Onaka et al., 2012). Additionally, a recent study found that formation of social reward was dependent on coordinated activity between oxytocin and serotonin in the mouse nucleus accumbens (Dölen et al., 2013). In rodents, the administration of oxytocin receptor agonist and exogenous synthetic oxytocin has also been found to increase social place preference and reduce social defeat induced avoidance (Hicks et al., 2012; Lukas et al., 2011). Conversely, the administration of oxytocin receptor antagonist, has been found to increase corticosteroid levels and induce social avoidance in monkeys during times of stress (Cavanaugh et al., 2016).

Recently there has been increasing interest in translating these findings into humans and the effects of intranasal oxytocin on social-emotional function has been widely studied (Bakermans-Kranenburg and van Ijzendoorn, 2013; Bartz et al., 2011; Guastella and MacLeod, 2012; Shahrestani et al., 2013; van Ijzendoorn and Bakermans-Kranenburg, 2012). A few previous meta-analytic reviews have found that intranasal oxytocin improves recognition of anger and happiness, and increases in-group trust among healthy individuals with small effect sizes (Shahrestani et al., 2013; van Ijzendoorn and Bakermans-Kranenburg, 2012). However, to our knowledge no meta-analyses to date have investigated the effects of a single dose of oxytocin on recognition of all six basic emotions, other aspects of emotion interpretation, including theory of mind or sensitivity to recognise basic emotions, or on emotion expression among healthy individuals.

To date, two meta-analytic reviews have investigated the effects of intranasal oxytocin on different aspects of social-emotional functioning in a variety of clinical groups (Bakermans-Kranenburg and van Ijzendoorn, 2013; Ooi et al., 2017). One reported small, but generally positive effect of intranasal oxytocin on social-emotional functioning and psychopathology among people with ASD, anxiety disorders, depression, schizophrenia, and BPD (Bakermans-Kranenburg and van Ijzendoorn, 2013). The other meta-analysis found no significant effects of intranasal oxytocin on social-emotional processing in ASD (Ooi et al., 2017). However, these reviews were quite heterogeneous pooling studies assessing psychopathology and social-emotional processing, or single dose and repeated dose studies into one meta-analysis (Bakermans-Kranenburg and van Ijzendoorn, 2013; Ooi et al., 2017). To our knowledge no previous meta-analyses have investigated the effects of a single dose of intranasal oxytocin separately on different aspects of interpretation and expression of emotions among both healthy and clinical populations. In order to consider the possibility of translating animal studies more widely into treatment for psychiatric disorders it is important to consider various key outcomes and whether there is evidence that they might be modified by oxytocin.

The aim of the current review was to pool studies investigating the effects of a single dose of intranasal oxytocin on various aspects of social-emotional functioning among healthy and clinical populations. Specifically, we aimed to examine the effects of intranasal oxytocin on theory of mind, recognition of basic emotions, sensitivity to recognise basic emotions, and on emotion expression among healthy and clinical populations. We tested the hypothesis that oxytocin would improve all aspects of social-emotional functioning.

2. Methodology

2.1. Literature searches

Electronic databases, including OVID (journals@OVID, PsycINFO, PsycARTICLES, Embase, AGRIS, MEDLINE), PubMed, and Web of Knowledge core collection, were searched for studies published during available years up to February 2017 in accordance with the PRISMA guidelines (Moher et al., 2009). Two separate literature searches were conducted in order to uncover studies investigating the effects of a single dose of intranasal oxytocin on interpretation and expression of emotions in a social context. The first literature search was conducted with the following search terms: *oxytocin AND emotion AND (interpretation OR recognition OR identification OR labelling OR "theory of mind" OR mentalising OR perception OR empath*)*. The second search was conducted with the following search terms: *oxytocin AND emotion AND (expression OR mimicry OR mirroring OR communication OR responsiveness OR expressivity)*. Additionally, to ensure no studies were missed by the initial search, the bibliographies of included studies were searched for additional studies.

2.2. Eligibility criteria

Studies were included if they met the following inclusion criteria: 1) investigated the effects of a single dose of intranasal exogenous oxytocin on interpretation or expression of emotions in a social context among either healthy adult participants or adult clinical populations (18 years old or older); 2) compared the effects of intranasal oxytocin with intranasal placebo spray; 3) investigated short term outcomes; and 4) randomly allocated participants to oxytocin and placebo groups or, in the case of crossover, within subjects studies, randomised the treatment order. Any studies, which used tasks that did not include a social component, such as the bumper car theory of mind task where social context is inferred from the movement of triangles on a computer screen, were excluded. Trials, in which participants either received repeated doses of oxytocin or in which long term outcomes of a single dose of oxytocin were assessed, were excluded. Studies that included only children or adolescents were excluded, because the majority of them were longer trials and the effects of oxytocin on social-emotional processing can be different in adults and children. Full-text articles published in peer reviewed journals and where possible, published conference abstracts were included.

In total, five studies were excluded after further screening because they incorporated tasks that were very different compared to the other included studies despite being otherwise relevant. These studies included a theory of mind task involving infant stimuli, continuously assessing the mood of a target on a video clip on a 9-point Likert scale, manipulating the context in which emotional stimuli was presented, recognising emotions from a point-light-display, and interpreting basic emotions from tone of voice in different languages (Bartz et al., 2010; Bernaerts et al., 2016; De Dreu et al., 2016; Perry et al., 2013; Voorthuis et al., 2014).

2.3. Study selection

The literature searches were conducted by one author (J.L.). The studies yielded from the literature search were then screened based on their titles and abstracts. Full text articles were then assessed for eligibility followed by final screening and assessment by two authors (J.L. and K.W.N.). Where appropriate conference abstracts of studies not yet published were also screened and assessed for eligibility. If deemed eligible the authors were contacted in order to gain access to the data. Only studies that both authors agreed on were included in the final systematic review and meta-analyses. Any cases where eligibility remained in question were brought to the whole team for further discussion and assessment. The study selection processes of the two

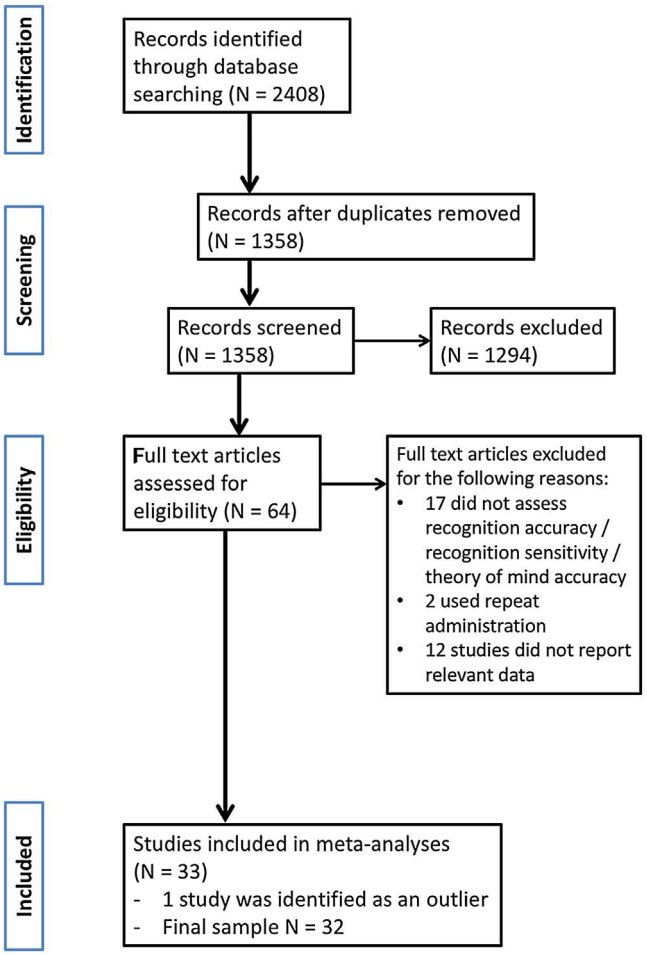

Fig. 1. PRISMA flow chart of the study selection process for the emotion interpretation meta-analyses.

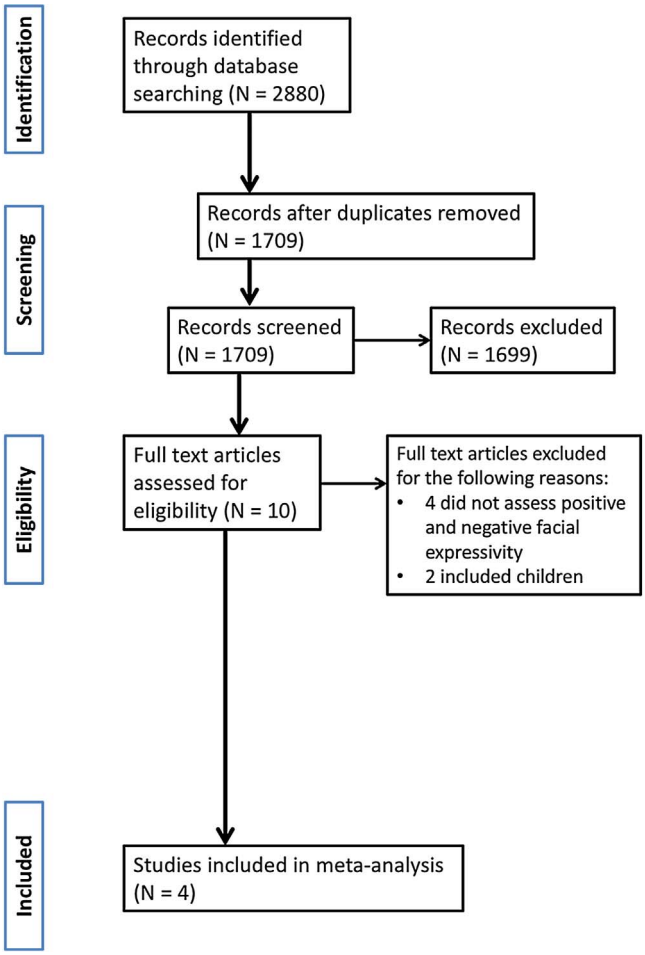

Fig. 2. PRISMA flow chart of the study selection process for the emotion expression meta-analyses.

searches are presented in Figs. 1 and 2.

2.4. Data collection and synthesis

Seventeen studies reported their results in figures or otherwise did not include the relevant data in the paper or supplementary materials. The corresponding authors of these papers were contacted by K.W.N. in order to gain access to the relevant data. The authors of the following papers provided the required data via personal correspondence: Aoki et al. (2014), Averbeck et al. (2012), Cardoso et al. (2014a), Chen et al. (2015), Fischer-Shofty et al. (2010), Kirkpatrick et al. (2014), Koch et al. (2016), Korb et al. (2016), and Luminet et al. (2011).

In order to conduct meta-analyses means, standard deviations, and samples sizes for both oxytocin and placebo groups or sessions were extracted from the studies or acquired through personal correspondence. Where standard error of the mean was reported, standard deviation was estimated with the following formula $SD = SE^{*}\sqrt{N}$. Altogether ten meta-analyses were conducted to investigate the effects of a single dose of intranasal oxytocin on interpretation and expression of emotions. The first meta-analysis investigated the effects of intranasal oxytocin on emotional theory of mind among healthy and clinical populations. The second meta-analysis investigated the effects of a single dose of intranasal oxytocin on interpretation of basic emotions among healthy and clinical populations. Five out of fifteen studies included in the overall basic emotion recognition meta-analysis included all six basic emotions. Thus, to provide further information about the effects of intranasal oxytocin on each of the six basic emotions, separate meta-analyses were conducted on recognition of anger, fear, disgust, sadness, surprise, and happiness among healthy and clinical populations. The last two meta-analyses investigated the effects of intranasal oxytocin on emotion recognition sensitivity and expression of congruent emotions in response to emotionally provoking stimuli. The clinical category included people with ASD, schizophrenia, depression, anorexia nervosa (AN), bulimia nervosa (BN), BPD, post-traumatic stress disorder, behavioural variant of frontotemporal dementia (FTD), alcohol dependence disorder, and opioid dependence disorder.

Additional information regarding age, the oxytocin dose (in international units [IU]), the specific diagnosis of clinical participants, the proportion of female participants in the sample, and type of task used were also recorded. We also included information regarding the presence of ceiling effects in accuracy scores. Since there are currently no guidelines to indicate what should be used as a cut-off for ceiling effects, we chose an arbitrary cut-off of 85%. Studies, in which the accuracy scores were greater than or equal to 85%, were coded as having evidence of ceiling effects. The additional information was used to conduct meta-regressions to identify variables that might explain any potential between-study heterogeneity.

2.5. Emotion interpretation tasks

The emotion interpretation tasks included are summarised in Table 1. The theory of mind tasks included the Reading the Mind in the Eyes task (RMET) (Baron-Cohen et al., 2001), Mayer-Salovey-Caruso Emotional Intelligence Test (MSCEIT) (Mayer et al., 2003), The Awareness of Social Inference Test (TASIT) (McDonald et al.,

Table 1
Study characteristics.

Study	Dose	Design	Dose-to-task interval	Group N	Age	Task	Scale	Emotion	ES [95% CI]	Power ≥80%
Healthy										
Bertsch et al. (2013)	26 IU	Between subjects	40 min	Healthy Oxytocin = 21 Placebo = 20	Oxytocin: 24.6 (3.9) Placebo: 24.4 (4.4)	Emotion recognition: Eyes	Accuracy	Anger	0.21 [−0.41, 0.82]	No
								Fear	0.37 [−0.25, 0.99]	
								Happiness	−0.03 [−0.65, 0.58]	
								Total	0.27 [−0.32, 0.87]	
						Emotion recognition: Mouth		Anger	0.41 [−0.21, 1.03]	
								Fear	0.33 [−0.29, 0.95]	
								Happiness	< 0.01 [−0.61, 0.61]	
								Total	0.23 [−0.31, 0.77]	
Brune et al. (2015)	24 IU	Within subjects	NR	Healthy N = 15	25.70 (6.40)	Ethological Coding System for Interviews	Expression	Affiliation	0.39 [−0.13, 0.92]	No
								Flight	−0.18 [−0.69, 0.33]	
Campbell et al. (2014)	20 IU	Between subjects	45 min	Older female N = 34	72.07 (6.49)	Emotion recognition	Accuracy	Total	−0.16 [−0.80, 0.48]	No
				Older male N = 34					0.77 [0.13, 1.40]	
				Younger female N = 34	19.68 (1.79)				0.07 [−0.54, 0.67]	
				Younger male N = 34					−0.01 [−0.62, 0.59]	
Cardoso et al. (2014)	24 IU	Between subjects	120 min	Healthy Oxytocin = 42 Placebo = 40	18–30	Mayer- Salovey-Caruso Emotional Intelligence Test (MSCEIT)	Accuracy	Understanding emotions (blends)	0.05 [−0.45, 0.54]	No
								Understanding emotions (changes)	0.08 [−0.42, 0.59]	
								Perceiving emotions (total faces)	−0.61 [−1.17, −0.06]	
Chen et al. (2015)	24 IU	Within subjects	45 min	Healthy N = 203	23.5 (2.7)	Dynamic emotion recognition	Accuracy	Anger	0.02 [−0.12, 0.16]	Yes
								Fear	0.13 [−0.01, 0.26]	
								Sadness	−0.05 [−0.19, 0.08]	
								Happiness	−0.11 [−0.25, 0.03]	
								Total	0.01 [−0.13, 0.15]	
							Recognition sensitivity	Anger	0.01 [−0.13, 0.15]	
								Fear	0.06 [−0.08, 0.20]	
								Sadness	0.15 [0.01, 0.29]	
								Happiness	0.05 [−0.09, 0.19]	
Domes et al. (2007)	24 IU	Within subjects	45 min	Healthy N = 20	24.3 (2.2)	RMET	Accuracy	Total	0.47 [0.01, 0.93]	No
Domes et al. (2014)	24 IU	Within subjects	45 min	Healthy N = 14	23.6 (5.4)	Emotion recognition: Eyes	Accuracy	Total	0.31 [−0.23, 0.84]	No
						Emotion recognition: Mouth			−0.25 [−0.79, 0.27]	
Feeser et al. (2014)	24 IU	Between subjects	45 min	Healthy Oxytocin = 41	37.9 (4.7)	Facial emotion recognition task	Accuracy	Anger	−0.31 [−0.50, −0.11]	No

(continued on next page)

Table 1 *(continued)*

Study	Dose	Design	Dose-to-task interval	Group N	Age	Task	Scale	Emotion	ES [95% CI]	Power ≥80%
				Placebo = 41						
								Fear	0.48 [0.29, 0.68]	
								Disgust	0.38 [−0.17, 0.94]	
								Sadness	0.23 [−0.33, 0.78]	
								Surprise	−0.17 [−0.73, 0.39]	
								Happiness	< 0.01 [−0.19, 0.19]	
								Total	0.60 [−0.16, 1.36]	
Feeser et al. (2015)	24 IU	Between subjects	45 min	Healthy Oxytocin = 36 Placebo = 35	Oxytocin = 27.2 (4.9) Placebo = 28.9 (4.8)	RMET	Accuracy	Total	0.62 [0.12, 1.12]	No
Fischer-Shofty et al. (2010)	24 IU	Within subjects	45 min	Healthy N = 27	26.93 (3.51)	Facial emotion recognition task	Accuracy	Anger	−0.05 [−0.43, 0.33]	No
								Fear	0.50 [0.10, 0.90]	
								Disgust	0.20 [−0.18, 0.58]	
								Sadness	−0.13 [−0.51, 0.25]	
								Surprise	−0.10 [−0.48, 0.28]	
								Happiness	0.01 [−0.37, 0.39]	
								Total	0.15 [−0.23, 0.53]	
Gamer et al. (2010)	24 IU	Between subjects	45 min	Healthy Oxytocin = 23 Placebo = 23	25.0 (3.7)	Facial emotion recognition task	Accuracy	Fear	−0.20 [−0.78, 0.38]	No
								Happiness	0.23 [−0.35, 0.81]	
								Total	0.06 [−0.52, 0.63]	
Kanat et al. (2015)	24IU	Between subjects	45 min	Healthy Oxytocin = 21 Placebo = 22	23.64 (2.81)	Masked emotion recognition task (17 ms)	Accuracy	Anger	−0.15 [−0.16, 0.96]	No
								Happiness	−0.05 [−0.65,0.55]	
								Total	−0.05 [90.65, 0.55]	
						Masked emotion recognition task (68 ms)		Anger	−0.32 [−0.74, 0.41]	
								Happiness	0.18 [−0.42, 0.77]	
								Total	−0.09 [−0.69, 0.50]	
Kim et al. (2015)	40 IU	Within subjects	45 min	Healthy N = 33	22.64 (2.28)	Dynamic emotion recognition	Recognition sensitivity	Anger	−0.26 [−0.60, 0.09]	No
								Fear	−0.23 [-0.58, 0.11]	
								Sadness	−0.41, −0.76, −0.05]	
								Happiness	0.03 [−0.63, 0.07]	
Kirkpatrick et al. (2014)	20 IU	Within subjects	30 min	Healthy N = 43	24.10 (4.10)	Dynamic emotion recognition	Accuracy	Anger	0.07 [−0.23, 0.36]	Yes
								Fear	−0.16 [−0.43, 0.17]	
								Sadness	0.02 [−0.25, 0.35]	
								Happiness	−0.24 [−0.55, 0.06]	
								Total	−0.05 [−0.35, 0.25]	

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Table 1 *(continued)*

Study	Dose	Design	Dose-to-task interval	Group N	Age	Task	Scale	Emotion	ES [95% CI]	Power ≥80%
	40 IU			Healthy N = 22	23.10 (3.50)			Anger	0.19 [−0.23, 0.61]	No
								Fear	0.37 [−0.06, 0.80]	
								Sadness	0.29 [−0.14, 0.71]	
								Happiness	0.20 [−0.22, 0.62]	
								Total	0.27 [−0.15, 0.70]	
Kirsch et al. (2005)	27 IU	Within subjects	50 min	Healthy N = 15	26.70 (3.00)	Emotion matching	Accuracy	Total	0.07 [−0.43, 0.58]	No
Koch et al. (2016)	40 IU	Within subjects	44.68 min	Trauma exposed healthy N = 40	40.00 (10.05)	Emotion matching	Accuracy	Total	−0.09 [−0.40, 0.22]	Yes
Korb et al. (2016)	24 IU	Between subjects	56 min	Healthy Oxytocin = 30 Placebo = 30	Oxytocin = 26.10 (5.10) Placebo = 23.60 (4.10)	Dynamic emotion: Happy to angry	Expression	EMG: CS	0.35 [−0.37, 1.07]	No
						Dynamic emotion: Angry to happy	Expression	EMG: ZM	0.15 [−0.39, 0.68]	
Leppanen et al. (2017)	40 IU	Within subjects	15 min	Healthy N = 29	26.83 (8.54)	RMET	Accuracy	Total	−0.21 [−0.58, 0.16]	No
						Evoked facial expressions to film stimuli	Expression	Happiness	0.22 [−0.14, 0.59]	
								Sadness	−0.24 [−0.61, 0.13]	
Lischke et al. (2012)	24 IU	Between subjects	45 min	Healthy Oxytocin = 23 Placebo = 24	Oxytocin = 25.78 (3.37) Placebo = 26.38 (3.49)	Dynamic emotion recognition	Accuracy	Anger	−0.16 [−0.70, 0.37]	No
								Fear	0.68 [0.24, 1.12]	
								Sadness	−0.04 [−0.47, 0.40]	
								Happiness	−0.03 [−0.57, 0.50]	
								Total	0.10 [−0.33, 0.52]	
							Recognition sensitivity	Anger	−0.77 [−1.25, −0.29]	
								Fear	−0.58 [−1.06, −0.09]	
								Sadness	−0.41 [−0.60, −0.21]	
								Happiness	−0.46 [−1.05, 0.13]	
								Total	−0.55 [−1.37, 0.03][b]	
Luminet et al. (2011)	32 IU	Between subjects	45 min	Healthy Oxytocin = 30 Placebo = 30	21.08 (2.13)	RMET	Accuracy	Total	0.30 [−0.32, 0.93]	No
Marsh et al. (2010)	24 IU	Between subjects	35 min	Healthy Oxytocin = 24 Placebo = 26	Oxytocin = 26.20 (4.90) Placebo = 26.60 (5.00)	Facial emotion recognition task	Accuracy	Anger	0.40 [−0.12, 0.93]	No
								Fear	0.32 [−0.28, 0.91]	
								Disgust	0.20 [−0.40, 0.80]	
								Sadness	−0.10 [−0.69, 0.49]	
								Surprise	0.48 [−0.07, 1.02]	
								Happiness	0.65 [0.12, 1.18]	
								Total	0.36 [−0.16, 0.89]	
Radke and de Bruijn	24 IU	Within	50–65 min	Healthy	21.50 (1.90)	RMET	Accuracy	Total	0.09 [−0.31, 0.49]	No

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Table 1 *(continued)*

Study	Dose	Design	Dose-to-task interval	Group N	Age	Task	Scale	Emotion	ES [95% CI]	Power ≥80%
(2015)		subjects		N = 24						
Riem et al. (2014)	16 IU	Between subjects	60 min	Healthy Oxytocin = 25 Placebo = 25	19.62 (1.47)	RMET	Accuracy	Total	−0.02 [−0.58, 0.55]	No
Schulze et al. (2011)	24 IU	Between subjects	45 min	Healthy Oxytocin = 28 Placebo = 28	24.18 (3.12)	Masked emotion recognition (mask: 18 ms)	Accuracy	Anger	0.53 [−0.03, 1.09]	No
								Happiness	0.66 [0.09, 1.23]	
								Total	0.29 [−0.33, 0.90]	
						Masked emotion recognition (mask: 35 ms)		Anger	0.47 [−0.11, 1.04]	
								Happiness	0.74 [0.16, 1.31]	
								Total	0.64 [−0.11, 1.38]	
						Masked emotion recognition (mask: 53 ms)		Anger	−0.02 [−0.45, 0.40]	
								Happiness	0.49 [0.07, 0.92]	
								Total	0.60 [−0.14, 1.35]	
Woolley et al. (2016)	40 IU	Within subjects	45 min	Healthy N = 33	51.91 (7.35)	RMET	Accuracy	Total	−0.06 [−0.40, 0.28]	No
Woolley et al. (2014)	40 IU	Within subjects	30 min	Healthy N = 31	42.50 (14.10)	TASIT: Emotion Evaluation Test	Accuracy	Total	0.04 [−0.32, 0.39]	No
						TASIT: Social Inference Enriched (feel)			−0.38 [−0.74, 0.01]	
						RMET			0.14 [−0.21, 0.50]	
Woolley et al. (2017)	40 IU	Within subjects	NR	Healthy N = 27	42.00 (13.70)	Evoked facial expressions to IAPS photos	Expression	Positive	0.26 [−0.12, 0.65]	No
								Negative	0.49 [0.09, 0.89]	
Xu et al. (2015)	40 IU	Between subjects	45 min	Healthy Oxytocin = 29 Placebo = 31	Oxytocin = 23.40 (0.30) Placebo = 22.90 (0.30)	RSVP task	Accuracy	Negative	0.76 [0.23, 1.28][a]	No
								Happiness	3.14 [2.39, 3.90][a]	
								Neutral	4.56 [3.60, 5.52][b]	
								Total	2.96 [2.23, 3.69][b]	
Clinical populations										
Aoki et al. (2015)	24 IU	Within subjects	40 min	ASD N = 20	30.8 (6.00)	Sally Anne: Social-emotional scale	Accuracy	Total	0.54 [0.07, 1.01]	No
Averbeck (2012)	24 IU	Within subjects	50 min	Schizophrenia N = 21	38.2 (1.8)	Hexagon emotion discrimination task: morphed faces 30% intensity	Accuracy	Anger	0.17 [−0.26, 0.60]	No
								Fear	0.11 [−0.32, 0.54]	
								Disgust	−0.06 [−0.49, 0.37]	
								Sadness	0.37 [−0.07, 0.81]	
								Surprise	< 0.01 [−0.43, 0.43]	
								Happiness	0.27 [−0.16, 0.71]	
								Total	0.58 [0.12, 1.05]	
						Hexagon emotion discrimination task: morphed faces 70% intensity		Anger	0.18 [−0.25, 0.61]	
								Fear	0.42 [−0.03, 0.87]	
								Disgust	0.13 [−0.30, 0.56]	
								Sadness	0.33 [−0.11, 0.77]	
								Surprise	−0.07 [−0.50, 0.36]	
								Happiness	0.16 [−0.27, 0.59]	

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Table 1 *(continued)*

Study	Dose	Design	Dose-to-task interval	Group N	Age	Task	Scale	Emotion	ES [95% CI]	Power ≥80%
								Total	0.58 [0.12, 1.05]	
Bertsch et al. (2013)	26 IU	Between subjects	40 min	BPD Oxytocin = 19 Placebo = 19	Oxytocin: 23.2 (5.3) Placebo: 24.9 (5.5)	Emotion recognition: Eyes	Accuracy	Anger	−0.09 [−0.73, 0.55]	No
								Fear	0.07 [−0.56, 0.71]	
								Happiness	−0.66 [−1.32, −0.01]	
								Total	−0.02 [−0.49, 0.44]	
						Emotion recognition: Mouth		Anger	0.39 [−0.25, 1.03]	
								Fear	−0.24 [−0.88, 0.39]	
								Happiness	−0.22 [−0.85, 0.42]	
								Total	−0.14 [−0.85, 0.58]	
Brune et al. (2015)	24 IU	Within subjects	NR	BPD N = 15	27.50 (7.30)	Ethological Coding System for Interviews	Expression	Affiliation	−0.22 [−0.73, 0.30]	No
								Flight	−0.17 [−0.68, 0.34]	
Domes et al. (2014)	24 IU	Within subjects	45 min	ASD N = 14	24.0 (6.9)	Emotion recognition: Eyes	Accuracy	Total	0.58 [0.02, 1.15]	No
						Emotion recognition: Mouth			0.53 [−0.03, 1.09]	
Guastella et al. (2015)	24 IU	Within subjects	45 min	Schizophrenia N = 21	37.42 (11.14)	Diagnostic Analysis of Non-Verbal Accuracy: Faces	Accuracy	Total	0.13 [−0.30, 0.56]	No
						Facial Expressions of Emotions Task			−0.40 [−0.84, 0.05]	
						RMET			0.39 [−0.05, 0.84]	
Jesso et al. (2011)	24 IU	Within subjects	20 min	FTD N = 20	64.4 (7.40)	RMET	Accuracy	Total	−0.46 [−0.92, 0.01]	No
Kim et al. (2015)	40 IU	Within subjects	45 min	AN N = 35	21.97 (8.41)	Dynamic emotion recognition	Recognition sensitivity	Anger	0.02 [−0.31, 0.36]	Yes
								Fear	0.14 [−0.19, 0.47]	
								Sadness	−0.01 [−0.34, 0.33]	
								Happiness	0.03 [−0.35, 0.31]	
				BN N = 34	23.03 (5.17)			Anger	−0.36 [−0.71, −0.01]	Yes
								Fear	−0.03 [-0.37, 0.31]	
								Sadness	−0.39 [−0.74, −0.04]	
								Happiness	0.03 [−0.58, 0.11]	
Koch et al. (2016)	40 IU	Within subjects	44.68 min	PTSD N = 36	39.93 (9.58)	Emotion matching	Accuracy	Total	0.36 [0.03, 0.70]	Yes
Leppanen et al. (2017)	40 IU	Within subjects	15 min	AN N = 30	26.2 (6.82)	RMET	Accuracy	Total	0.10 [−0.26, 0.46]	No
						Evoked facial expressions to film stimuli	Expression	Happiness	−0.04 [−0.40, 0.31]	
								Sadness	−0.40 [−0.77, −0.03]	
MacDonald et al. (2013)	40 IU	Within subjects	75 min	Depression N = 17	43.65 (12.20)	RMET	Accuracy	Total	0.50 [−0.01, 1.00]	No
Mitchell et al. (2016)	40 IU	Within subjects	30 min	Alcohol dependence N = 32	28.90 (7.15)	RMET	Accuracy	Total	0.24 [−0.12, 0.60]	No

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Table 1 *(continued)*

Study	Dose	Design	Dose-to-task interval	Group N	Age	Task	Scale	Emotion	ES [95% CI]	Power ≥ 80%
Woolley et al. (2016)	40 IU	Within subjects	45 min	Opioid dependence N = 33	57.97 (8.88)	RMET	Accuracy	Total	−0.51 [−0.88, −0.15]	No
Woolley et al. (2014)	40 IU	Within subjects	30 min	Schizophrenia N = 29	44.60 (10.70)	TASIT: Emotion Evaluation Test	Accuracy	Total	−0.02 [−0.38, 0.35]	No
						TASIT: Social Inference Enriched (feel)			0.35 [−0.02, 0.73]	
						RMET			−0.02 [−0.38, 0.35]	
Woolley et al. (2017)	40 IU	Within subjects	NR	Schizophrenia N = 25	43.20 (11.00)	Evoked facial expressions to IAPS photos	Expression	Positive	0.23 [−0.17, 0.63]	No
								Negative	0.33 [−0.07, 0.73]	

ES = effect size; IU = international unit; NR = not reported; AN = anorexia nervosa, ASD = autism spectrum disorder, BN = bulimia nervosa, BPD = borderline personality disorder, PTSD = posttraumatic stress disorder RMET = reading the mind in the eyes test; TASIT = The Awareness of Social Inference Test; IAPS = International Affective Picture System. "No" in the power column indicates the study did not reach the sample size requirement for adequate statistical power and "Yes" in the power column indicates the study reached the sample size requirement for adequate statistical power.

[a] Influential outlier. Excluded from meta-analysis.

[b] Not included in meta-analysis.

2003), and the Sally Anne task (Baron-Cohen et al., 1985). In the RMET participants are presented with 36 photographs of eyes and asked to select one of four words that best describes the complex emotion displayed. The MSCEIT has six subscales, three of which were of interest for the current review and assessed perception of emotions in others and understanding of how emotions change and blend together. The TASIT has three subscales, two of which were of interest for the current review and assessed emotional evaluation and social-emotional inference based on verbal and visual stimuli. Finally, the Sally Anne task assesses emotional and cognitive theory of mind with black and white comic strips. The current reviewed only included data on emotional theory of mind.

The studies that investigated the effects of a single dose of intranasal oxytocin on interpretation of basic emotions mostly used standard emotion recognition paradigms in which participants were presented with a photograph of a face depicting a basic emotion and asked to identify the emotion. A subset of studies used dynamic emotion recognition tasks, in which participants were presented with a neutral face that gradually morphed into the full emotion, or masked emotion recognition tasks, in which the photograph of a face depicting a basic emotion was either preceded or followed by a neutral face. The outcome was recognition accuracy. All studies used standardised stimuli to assess emotion recognition accuracy.

All studies examining interpretation sensitivity used similar dynamic emotion recognition tasks as described above. In these tasks participants were initially presented with a neutral face that gradually morphed into full emotion. The outcome was estimated as the intensity percentage at which participants accurately recognised the emotion. Lower the intensity percentage, the more sensitive the participants were to detect the emotion. All studies used standardised stimuli to assess emotion recognition sensitivity.

2.6. Emotion expression tasks

The emotion expression tasks included are summarised in Table 1. All included studies used different paradigms to elicit positive and negative emotions. One of the studies presented participants with emotionally provoking positive and negative images from the International Affective Picture System (IAPS). Two of the studies presented participants with short film clips. In one study the film clips depicted either a happy face that gradually morphed into an angry expression or an angry face that gradually morphed into a happy expression. The other study presented participants with excerpts from movies that were designed to elicit happy and sad emotions. Lastly, one study assessed expressions of flight and affiliation during a clinical interview. Participants facial expression were recorded either by manually assessing facial expression using the Facial Expression Coding System (FACES, (Kring and Sloan, 1991)), using facial electromyography (EMG), or using automated facial emotion detection software, Noldus FaceReader (Noldus Information Technology b.v., www.noldus.com).

2.7. Statistical analysis

All statistical analysis was performed using R (R Core Team, 2015). For studies using between subjects design Hedges' g was calculated to estimate unbiased effect size with 95% confidence intervals. For studies using within subjects design standardised mean change (SMC) with change score standardisation was calculated to estimate effect size with 95% confidence intervals. The SMC controls for the correlation in task performance between the two assessments (oxytocin session and placebo session). Where correlation between the two assessments was not reported, the correlation coefficient was estimated using the following formula $r = \frac{SD1^2 + SD2^2 - SDchange^2}{2 \times SD1 \times SD2}$(Morris and DeShon, 2002). Both Hedges' g and SMC effect size estimates are on the same scale and were interpreted as small (≥0.20), medium (≥0.50), and large

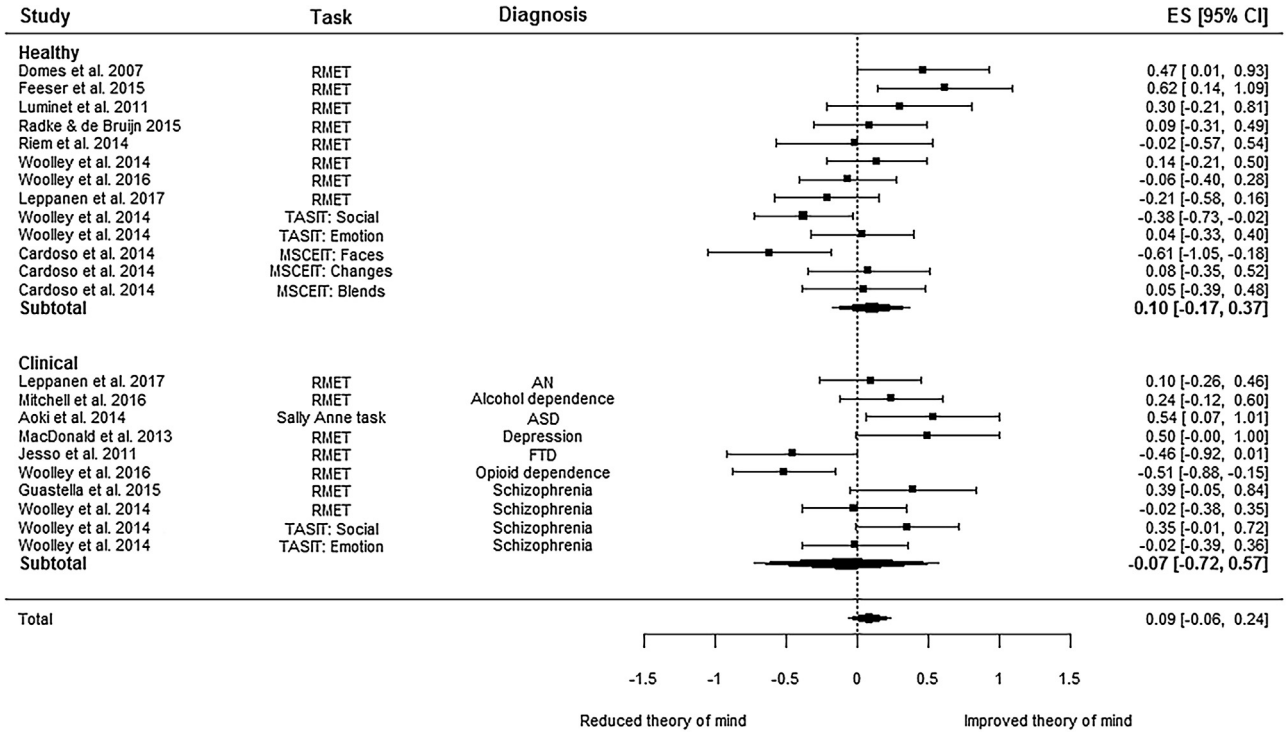

Fig. 3. Effects of intranasal oxytocin vs. placebo on theory of mind. Positive effect sizes indicate improved theory of mind following oxytocin administration; negative effect sizes indicate reduced theory of mind following oxytocin administration. RMET = Reading the mind in the eyes test; MSCEIT = Mayer-Salovey-Caruso Emotional Intelligence Test; TASIT = The Awareness of Social Inference Test; AN = anorexia nervosa; ASD = autism spectrum disorder; frontotemporal dementia = FTD.

(≥0.80) (Hedges, 1981). In nine of the ten meta-analyses higher scores indicated either greater accuracy or greater facial expressivity. Thus, in these meta-analyses positive effect size indicated improved emotion interpretation accuracy or increased emotion expression following oxytocin administration, whereas negative effect size indicated poorer emotion interpretation or reduced emotion expression following oxytocin administration. In the meta-analysis investigating emotion recognition sensitivity lower scores indicated greater sensitivity to recognise the facial expressions. Thus, in this meta-analysis negative effect sizes indicated increased sensitivity to recognise emotions following oxytocin administration and positive effect sizes indicated reduced sensitivity to recognise emotions following oxytocin administration. Significance threshold was set at $p < 0.05$ unless otherwise stated.

The user contributed Metafor package was used to conduct the meta-analyses, meta-regressions, and publication bias estimation (Viechtbauer, 2010). The meta-analyses were conducted with a multivariate random effects model with an autoregressive structure using the *rma.mv* function in Metafor to account for correlations arising from multiple outcomes from the same sample. Between-study heterogeneity was assessed by calculating *Cochran's Q* index and I^2 index. Where between-study heterogeneity was found meta-regressions were conducted and the moderator effects of the following variables was assessed: age, the dose administered (in IU), the specific diagnosis of clinical participants, the type of task used, the proportion of female participants in the sample, and whether there were ceiling effects present (accuracy ≥ 85%). All binary and categorical moderators were dummy coded and entered into the meta-regression using the *factor* function. The impact of each moderator was assessed in separate models.

Influential studies and extreme outliers were identified by inspecting Cook's distance plots and the standardised residuals of each individual study. Where the z-score of the standardised residuals exceeded 1.96, the study was deemed to be an outlier (Viechtbauer and Cheung, 2010). To test the impact of any outliers on the pooled effect size estimate, we conducted the meta-analysis with the outlier present followed by a meta-regression to examine whether the outlier significantly explained the between study heterogeneity. If the outlier significantly explained the heterogeneity, the outlier was excluded and only results from the meta-analysis without the outlier were reported in full. This procedure led to exclusion of one study (Xu et al., 2015) from two separate meta-analyses.

Publication bias was investigated with Begg's rank correlation test for funnel plot asymmetry (Begg and Mazumdar, 1994) and where significant effects were present Rosenthal's file drawer analysis was also conducted (Rosenthal, 1979). The robustness of the significant findings was assessed by calculating the Rosenthal's criterion ($5n + 10$, n = the number of studies in the meta-analysis) and comparing that figure to the fail-safe N from the file drawer analysis. If the fail-safe N exceeded the criterion the findings were considered robust, if it did not this was taken as an indicator of publication bias.

Finally, the statistical power of each study included in the review was assessed by comparing the sample sizes in these studies against that recommended by a power calculation conducted with G*Power (Faul et al., 2007). According to the power calculation studies using between subjects design should have at least 64 participants in each group while studies using within subjects design should have at least 34 participants altogether, to have adequate statistical power (≥80%) to reliably detect a moderate difference (ES ≥ 0.5) between the two groups or conditions.

3. Results

3.1. Study characteristics

The characteristics of the 33 included studies are summarised in Table 1. The effect size estimate (ES) represents standardised mean difference (Hedges' g) or standardised mean change (SMC) in interpretation and expression of emotions following oxytocin and placebo. The last column in Table 1 indicates whether the study met the sample size requirement for adequate statistical power (≥80%) to reliably

detect at least a medium sized effect between the oxytocin and placebo groups or conditions. None of the studies that used between subjects design met the requirement for adequate power, but five of the studies that used within subjects design did meet this requirement.

3.2. Effects of oxytocin on interpretation of emotions

3.2.1. Theory of mind

Fourteen studies were included in the meta-analysis investigating the effects of a single dose of intranasal oxytocin on emotional theory of mind (Fig. 3). Six of the studies included clinical populations, namely individuals with ASD, AN, depression, schizophrenia, and FTD, and two studies included individuals with substance dependence disorder, including opioid and alcohol dependence. Overall, there was no significant effect of oxytocin on theory of mind (ES = 0.09, Z = 1.14, p = 0.256, 95% CI [−0.06, 0.24], k = 23, N of levels = 17). When the healthy and clinical groups were inspected separately there was no evidence of significant effect of intranasal oxytocin on emotional theory of mind within either group (Healthy: ES = 0.07, Z = 0.76, p = 0.447, 95% CI [-0.10, 0.24], k = 13, N of levels = 9; Clinical: ES = 0.10, Z = 0.74, p = 0.457, 95% CI [-0.17, 0.37], k = 10, N of levels = 8).

There was evidence of significant between-study heterogeneity (Q = 65.85, p < 0.0001, I^2 = 58.42%), which was further explored with meta-regressions. The findings from the meta-regressions were corrected for multiple comparisons with Bonferroni correction (0.05/6) and p < 0.008 was considered significant. The meta-regressions revealed a significant moderator effect of age (Qm = 7.33, p = 0.007; Supplementary Fig. 1), still leaving some residual heterogeneity (Qr = 34.26, p = 0.012). This finding suggests that younger participants showed greater oxytocin-induced improvement in emotional theory of mind. The dose administered (Qm = 1.04, p = 0.309; Supplementary Fig. 2), the type of task used (Qm = 2.78, p = 0.427), the diagnostic group (Qm = 0.01, p = 0.918), and the proportion of female participants in the sample (Qm = 1.36, p = 0.243; Supplementary Fig. 3) did not significantly explain the between-study heterogeneity.

Begg's rank correlation test of funnel plot asymmetry approached significance suggesting there may have been some publication bias (T = 0.29, p = 0.057; Supplementary Fig. 4).

3.2.2. Recognition of basic emotions

Seventeen studies investigated the effects of intranasal oxytocin on total basic emotion recognition accuracy. Based on standardised residuals and Cook's distance, one study by Xu et al. (2015) was identified as an extreme outlier (standardised res. = 2.68, Z = 4.56, SE = 0.59; Supplementary Table 1; Supplementary Fig. 5). The impact of the outlier on the meta-analysis was investigated by conducting a meta-analysis with the outlier present, which yielded a significant oxytocin-induced improvement in recognition of basic emotions with a small effect size along with significant between-study heterogeneity (ES = 0.28, Z = 2.56, p = 0.011, 95% CI [0.07, 0.50], k = 33, N of levels = 23, Q = 99.98, p < 0.0001, I^2 = 81.12%). We then investigated whether the outlier was significantly different from the other studies by conducting a meta-regression, which revealed that the outlier significantly explained the between-study heterogeneity (Qm = 46.98, p < 0.0001) leaving no significant residual heterogeneity (Qr = 43.51, p = 0.067). Therefore, the outlier was removed from further analysis.

Full meta-analysis was conducted with the remaining sixteen studies (Fig. 4). Five of the studies included clinical populations, namely people with schizophrenia, BPD, ASD, and post-traumatic stress disorder. The meta-analysis revealed that intranasal oxytocin administration improved overall basic emotion interpretation accuracy with a negligible effect size (ES = 0.18, Z = 3.09, p = 0.002, 95% CI [0.06, 0.29], k = 32, N of levels = 22). When the effects of intranasal oxytocin on basic emotions recognition were further investigated within the healthy and clinical populations, the results showed that oxytocin significantly improved basic emotion recognition among the healthy individuals with a negligible effect size (ES = 0.13, Z = 2.30, p = 0.022, 95% CI [0.02, 0.24], k = 23, N of levels = 17). Among the mixed clinical population oxytocin-induced improvement on basic emotion recognition approached significance with a small effect size (ES = 0.27, Z = 1.82, p = 0.069, 95% CI [-0.02, 0.57], k = 9, N of levels = 5).

The between-study heterogeneity approached significance (Q = 42.08, p = 0.089, I^2 = 30.27%), and was therefore, explored further with meta-regressions. The findings from the meta-regressions were corrected for multiple comparisons with Bonferroni correction (0.05/7) and p < 0.007 was considered significant. The proportion of female participants in the sample (Qm = 1.34, p = 0.247; Supplementary Fig. 6), the diagnostic group (Qm = 3.47, p = 0.482), age (Qm = 0.67, p = 0.414; Supplementary Fig. 7), the dose administered (Qm = 0.49, p = 0.486; Supplementary Fig. 8), the type of task used (Qm = 1.32, p = 0.858), and the presence of ceiling effects (Qm = 1.48, p = 0.224) did not significantly explain the between-study heterogeneity.

Begg's rank correlation test of funnel plot asymmetry did not reveal significant publication bias (T = −0.08, p = 0.506; Supplementary Fig. 9). Rosenthal's the file drawer analysis indicated a fail-safe N of 182, suggesting that 182 studies finding no significant effects of oxytocin on recognition of basic emotions would be required to reduce the observed effects to null. This exceeds Rosenthal's criterion for this meta-analysis ($5n+10=90$) suggesting that the effect was quite robust.

Since the total scores in most of the included studies did not consist of all six basic emotions further meta-analyses were conducted to examine if he above effect was driven by a subset of basic emotions. Thus, we conducted six additional meta-analyses to investigate the effects of a single dose of intranasal oxytocin on the recognition of anger, fear, disgust, sadness, surprise, and happiness. Where possible the studies were divided into healthy and clinical subgroups, which were both inspected separately.

3.2.3. Recognition of anger

Ten studies investigated the effects of intranasal oxytocin on recognition of anger (Fig. 5). Two of these studies included people with schizophrenia and BPD.

Overall, oxytocin administration did not significantly improve recognition of anger (ES = 0.05, Z = 1.03, p = 0.305, 95% CI [−0.05, 0.15], k = 18, N of levels = 11). When the groups were inspected separately, there was no significant effect of oxytocin on recognition of anger within the healthy (ES = 0.04, Z = 0.71, p = 0.476, 95% CI [−0.07, 0.14], k = 14, N of levels = 9) or clinical groups (ES = 0.17, Z = 1.10, p = 0.275, 95% CI [−0.13, 0.47], k = 4, N of levels = 2).

There was no evidence of significant between-study heterogeneity (Q = 21.16, p = 0.219, I^2 = 3.45e-09%) There was also no evidence of significant publication bias on the Begg's rank correlation test of funnel plot asymmetry (T = 0.03, p = 0.881; Supplementary Fig. 10).

3.2.4. Recognition of fear

Nine studies investigated the effects of intranasal oxytocin on recognition of fear (Fig. 6). Two of the studies included people with schizophrenia and BPD.

Overall, oxytocin administration significantly improved recognition of fear with a small effect size (ES = 0.21, Z = 2.95, p = 0.003, 95% CI [0.07, 0.34], k = 14, N of levels = 10). This effect was driven by a significant oxytocin-induced improvement in recognition of fear among the healthy individuals with a small effect size (ES = 0.24, Z = 2.63, p = 0.009, 95% CI [0.06, 0.41], k = 10, N of levels = 8). There was no significant effect of oxytocin among the mixed clinical population (ES = 0.16, Z = 1.05, p = 0.295, 95% CI [-0.14, 0.46]).

The between-study heterogeneity approached significance (Q = 19.95, p = 0.096, I^2 = 20.92%) and was therefore explored

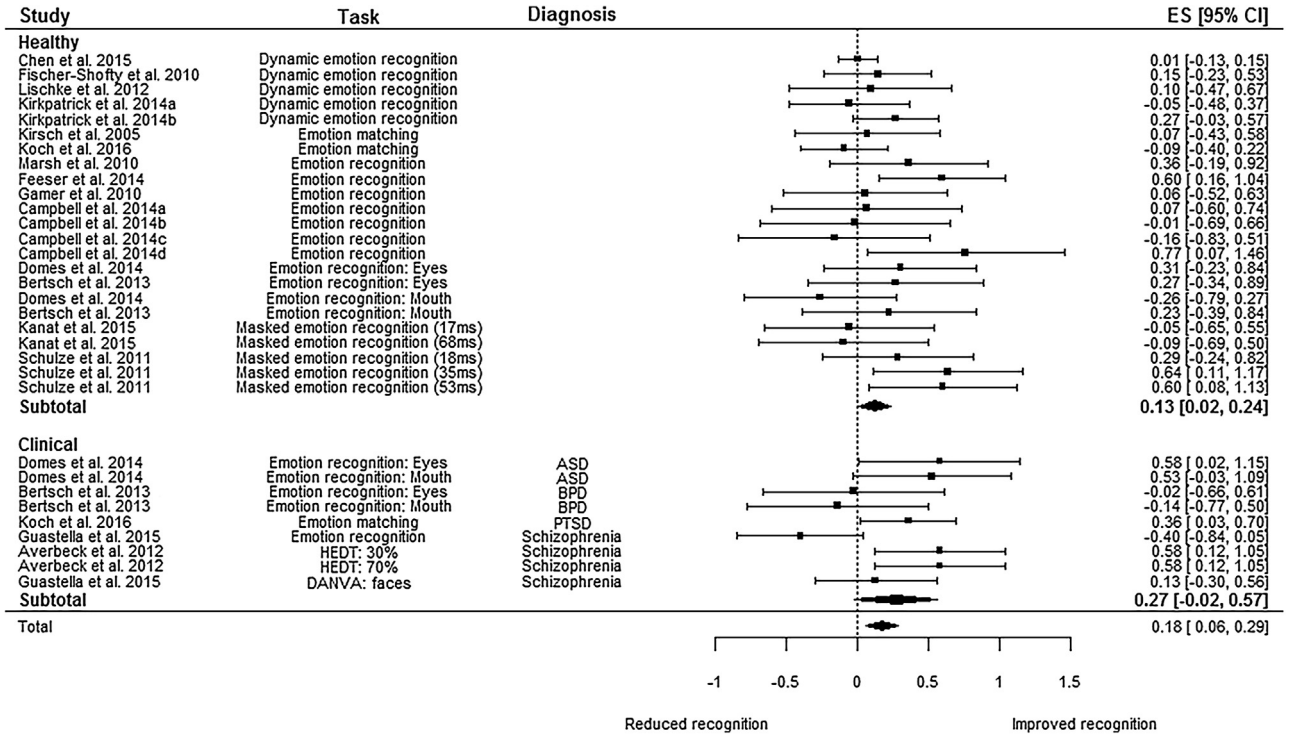

Fig. 4. Effect of oxytocin vs. placebo on overall basic emotion recognition. Positive effect sizes indicate improved emotion recognition following oxytocin administration; negative effect sizes indicate reduced emotion recognition following oxytocin administration. HEDT: 30% = Hexagon emotion discrimination task: morphed faces 30% intensity; HEDT: 70% = Hexagon emotion discrimination task: morphed faces 70% intensity; DANVA = Diagnostic Analysis of Non-Verbal Accuracy; ASD = Autism spectrum disorder; BPD = Borderline personality disorder; PTSD = Post-traumatic stress disorder. Kirkpatrick et al., 2014: a = 20IU of intranasal oxytocin, b = 40IU of intranasal oxytocin. Campbell et al., 2014: a = young female participants, Campbell et al., 2014: b = young male participants, Campbell et al., 2014: c = older female participants, Campbell et al., 2014: d = older male participants.

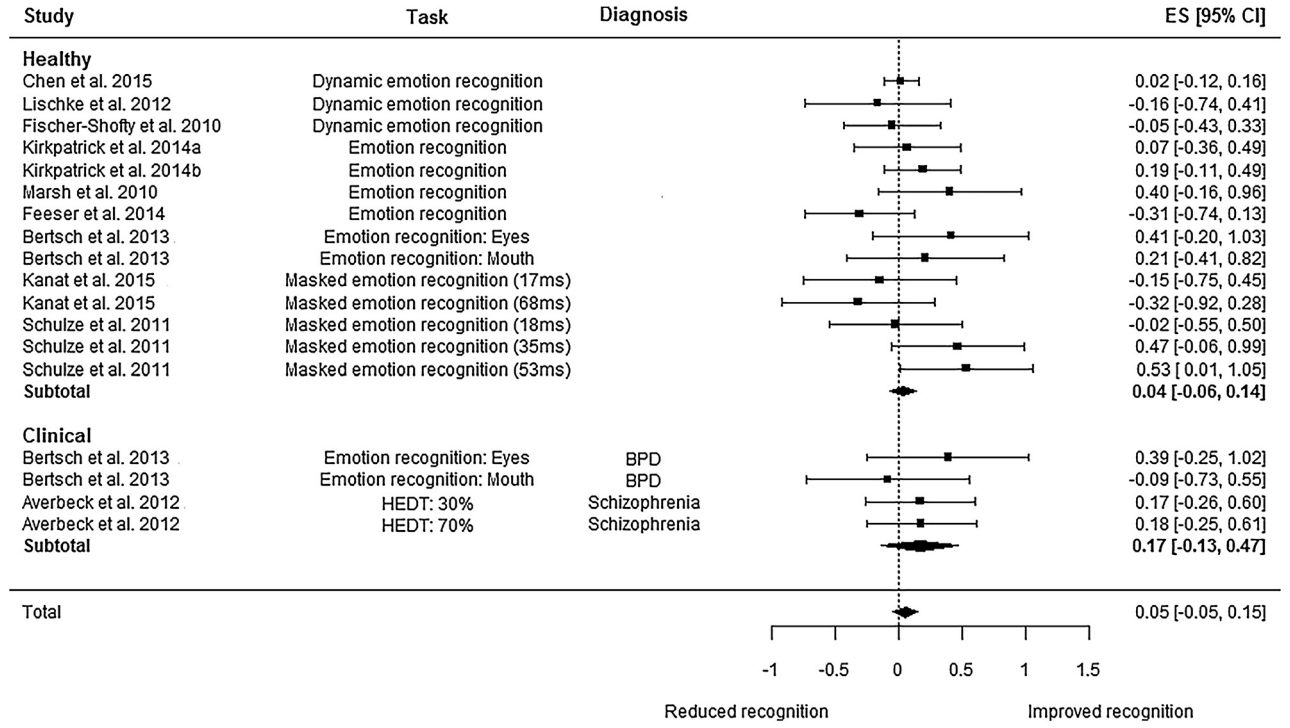

Fig. 5. Effect of oxytocin vs. placebo on recognition of anger. Positive effect sizes indicate improved recognition of anger following oxytocin administration; negative effect sizes indicate reduced recognition of anger following oxytocin administration. HEDT: 30% = Hexagon emotion discrimination task: morphed faces 30% intensity; HEDT: 70% = Hexagon emotion discrimination task: morphed faces 70% intensity; BPD = Borderline personality disorder. Kirkpatrick et al., 2014: a = 20IU of intranasal oxytocin, b = 40IU of intranasal oxytocin.

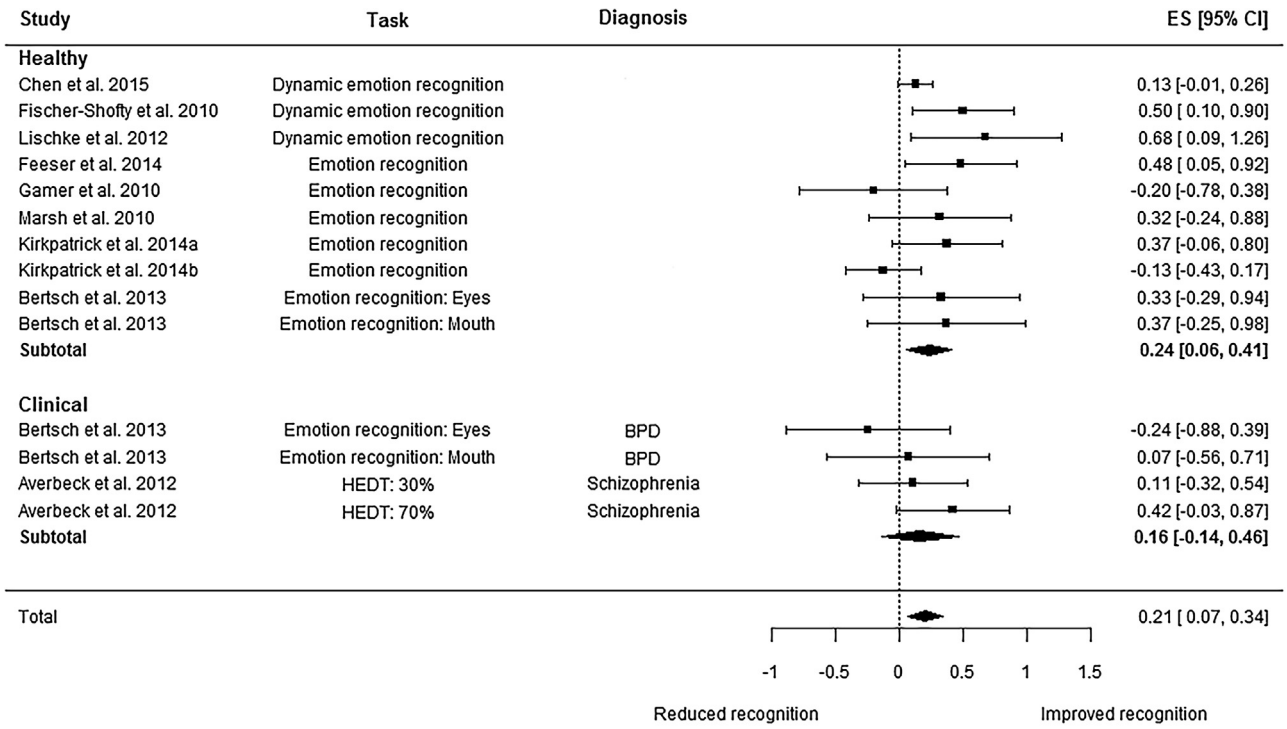

Fig. 6. Effects of oxytocin vs. placebo on recognition of fear. Positive effect sizes indicate improved recognition of fear following oxytocin administration; negative effect sizes indicate reduced recognition of fear following oxytocin administration. HEDT: 30% = Hexagon emotion discrimination task: morphed faces 30% intensity; HEDT: 70% = Hexagon emotion discrimination task: morphed faces 70% intensity.

further with meta-regressions. The findings from the meta-regressions were corrected for multiple comparisons with Bonferroni correction (0.05/7) and $p < 0.007$ was considered significant. The dose administered ($Qm = 4.19$, $p = 0.041$; Supplementary Fig. 11), the proportion of female participants in the sample ($Qm = 0.23$, $p = 0.632$; Supplementary Fig. 12), the presence of ceiling effects ($Qm = 0.05$, $p = 0.831$), age ($Qm = 2.56$, $p = 0.109$; Supplementary Fig. 13), the type of task used ($Qm = 0.78$, $p = 0.676$), and the diagnostic group ($Qm = 0.90$, $p = 0.638$) did not significantly explain the between-study heterogeneity.

There was no evidence of significant publication bias on the Begg's rank correlation test of funnel plot asymmetry ($T = -0.08$, $p = 0.747$; Supplementary Fig. 14). Rosenthal's file drawer analysis revealed a fail-safe N of 59, suggesting that 59 additional studies reporting no effect of oxytocin would be needed to reduce the observed effect to null. This exceeds Rosenthal's criterion for this meta-analysis ($5n+10 = 55$), suggesting the effect was quite robust.

3.2.5. Recognition of disgust

Four studies investigated the effects of oxytocin on the recognition of disgust (Fig. 7). Because only one of these studies included clinical populations, namely people with schizophrenia, it was not possible to investigate the effects of oxytocin separately within the healthy and clinical populations. Thus, the data was analysed across groups.

The meta-analysis showed a negligible oxytocin-induced improvement in the recognition of disgust, which approached significance (ES = 0.18, Z = 1.73, p = 0.083, 95% CI [-0.02, 0.39], k = 5, N of levels = 4).

There was no evidence of significant between-study heterogeneity ($Q = 2.07$, $p = 0.722$, $I^2 = 2.73e\text{-}08\%$).

There was no evidence of significant publication bias on the Begg's rank correlation test of funnel plot asymmetry ($T = 0.20$, $p = 0.817$; Supplementary Fig. 15).

3.2.6. Recognition of sadness

Seven studies investigated the effects of oxytocin on the recognition of sadness (Fig. 8). As above, since only one study included a clinical group, people with schizophrenia, the meta-analysis was conducted across groups.

The meta-analysis revealed no significant effects of oxytocin on the recognition of sadness (ES = 0.04, Z = 0.53, p = 0.594, 95% CI [-0.10, 0.17]). There was also no significant between-study heterogeneity ($Q = 7.59$, $p = 0.474$, $I^2 = 16.67\%$).

There was no evidence of significant publication bias on the Begg's rank correlation test of funnel plot asymmetry ($T = 0.33$, $p = 0.260$; Supplementary Fig. 16).

3.2.7. Recognition of surprise

Four studies investigated the effects of oxytocin on the recognition of surprise (Fig. 9). Since only one study included a clinical group, people with schizophrenia, the meta-analysis was conducted across groups.

The meta-analysis revealed no significant effect of oxytocin on the recognition of surprise (ES = −0.02, Z = −0.16, p = 0.874, 95% CI [-0.22, 0.19], k = 5, N of levels = 4). There was also no evidence of significant between-study heterogeneity ($Q = 3.72$, $p = 0.445$, $I^2 = 1.90e\text{-}08\%$)

There was no evidence of significant publication bias on Begg's rank correlation test of funnel plot asymmetry ($T = 0.20$, $p = 0.817$; Supplementary Fig. 17).

3.2.8. Recognition of happiness

Twelve studies investigated the effects on intranasal oxytocin on the recognition of happiness. Following inspection of the standardised residuals and Cook's distance one study by Xu et al. (2015) was identified as an extreme outlier (standardised res. = 2.83, Z = 3.27, SE = 0.87; Supplementary Table 2, Supplementary Fig. 18). The meta-analysis with the outlier present yielded no significant effect of oxytocin on the recognition of happiness, but there was significant between-study heterogeneity (ES = 0.31, Z = 1.26, p = 0.207, 95% CI [-0.17, 0.79], k = 20, N of levels = 12, $Q = 93.93$, $p < 0.0001$, $I^2 = 93.34\%$). A meta-regression revealed that the outlier significantly

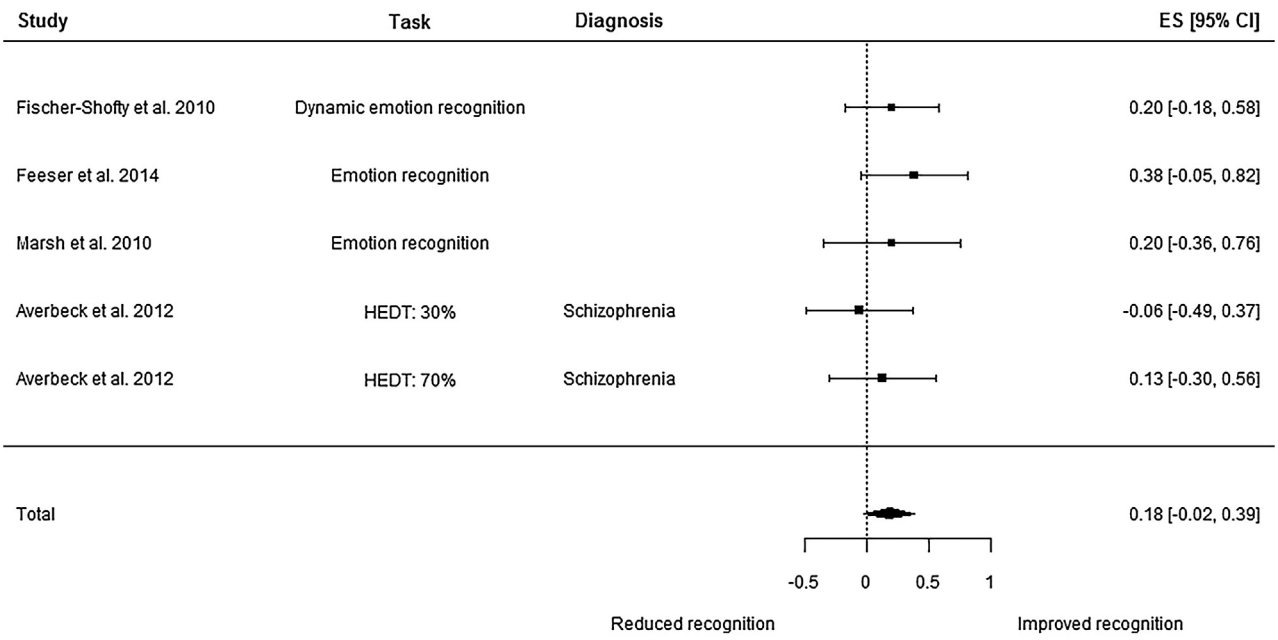

Fig. 7. Effect of oxytocin vs. placebo on recognition of disgust. Positive effect sizes indicate improved recognition of disgust following oxytocin administration; negative effect sizes indicate reduced recognition of disgust following oxytocin administration. HEDT: 30% = Hexagon emotion discrimination task: morphed faces 30% intensity; HEDT: 70% = Hexagon emotion discrimination task: morphed faces 70% intensity; BPD = Borderline personality disorder. Kirkpatrick et al., 2014: a = 20IU of intranasal oxytocin, b = 40IU of intranasal oxytocin.

explained the between-study heterogeneity (Qm = 51.44, p < 0.0001) leaving no significant residual heterogeneity (Qr = 28.36, p = 0.057). Therefore, the outlier was removed from further analysis.

The eleven remaining studies were included in the final meta-analysis (Fig. 10). Two of the studies included clinical groups, people with schizophrenia and BPD. Overall, oxytocin did not significantly improve the recognition of happiness (ES = 0.08, Z = 1.07, p = 0.284, 95% CI [-0.07, 0.23], k = 19, N of levels = 12). When the healthy and clinical groups were inspected separately, there were no differential effects among the healthy (ES = 0.10, Z = 1.18, p = 0.237, 95% CI [-0.06, 0.26], k = 15, N of levels = 10), or mixed clinical populations (ES = −0.07, Z = −0.23, p = 0.821, 95% CI [-0.72, 0.57]).

The between-study heterogeneity approached significance (Q = 27.87, p = 0.064, I^2 = 33.62%) and was explored further with meta-regressions. The findings from the meta-regressions were corrected for multiple comparisons with Bonferroni correction (0.05/7) and p < 0.007 was considered significant. The type of task used (Qm = 11.36, p = 0.010), the proportion of female participants in the sample (Qm = 0.84, p = 0.360; Supplementary Fig. 19), age (Qm = 0.07, p = 0.789; Supplementary Fig. 20), the dose administered

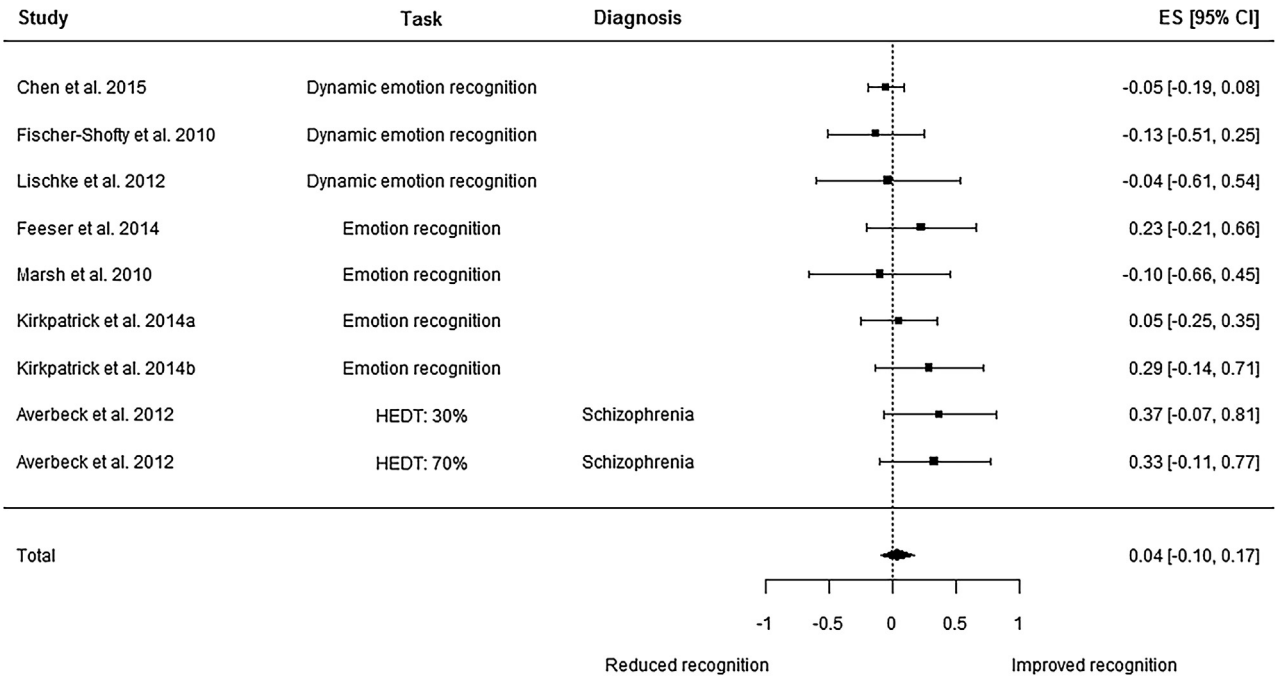

Fig. 8. Effect of oxytocin vs. placebo on recognition of sadness. Positive effect sizes indicate improved recognition of sadness following oxytocin administration; negative effect sizes indicate reduced recognition of sadness following oxytocin administration. HEDT: 30% = Hexagon emotion discrimination task: morphed faces 30% intensity; HEDT: 70% = Hexagon emotion discrimination task: morphed faces 70% intensity. Kirkpatrick et al., 2014: a = 20IU of intranasal oxytocin, b = 40IU of intranasal oxytocin.

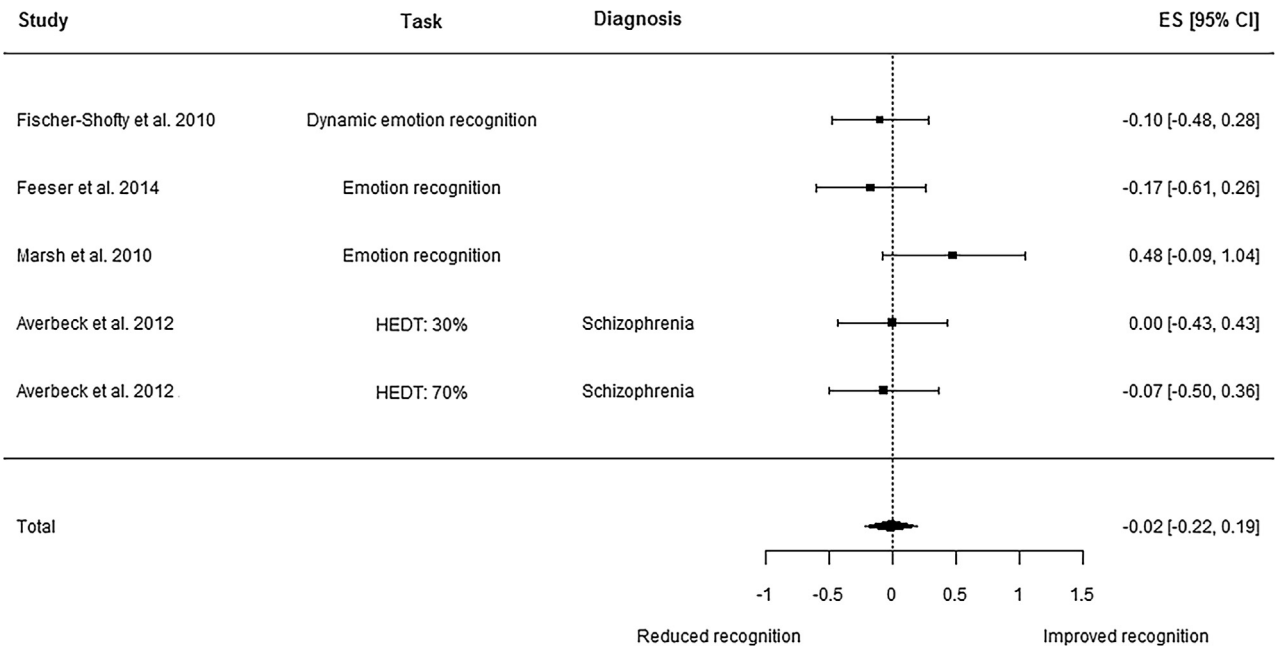

Fig. 9. Effect of oxytocin vs. placebo on recognition of surprise. Positive effect sizes indicate improved recognition of surprise following oxytocin administration; negative effect sizes indicate reduced recognition of surprise following oxytocin administration. HEDT: 30% = Hexagon emotion discrimination task: morphed faces 30% intensity; HEDT: 70% = Hexagon emotion discrimination task: morphed faces 70% intensity.

(Qm = 2.67, p = 0.103; Supplementary Fig. 21), the diagnostic group (Qm = 2.84, p = 0.242), and the presence of ceiling effects (Qm = 3.29, p = 0.070), did not significantly explain the between-study heterogeneity.

There was no evidence of significant publication bias on the Begg's rank correlation test of funnel plot asymmetry (T = −0.13, p = 0.447; Supplementary Fig. 22).

3.2.9. *Emotion recognition sensitivity*

Three studies investigated the effects of intranasal oxytocin on emotion recognition sensitivity (Fig. 11). Because only one of these studies included clinical groups, people with AN and BN, the meta-analysis was conducted across groups.

Overall, intranasal oxytocin did not significantly influence emotion recognition sensitivity (ES = −0.14, Z = −1.45, p = 0.146, 95% CI

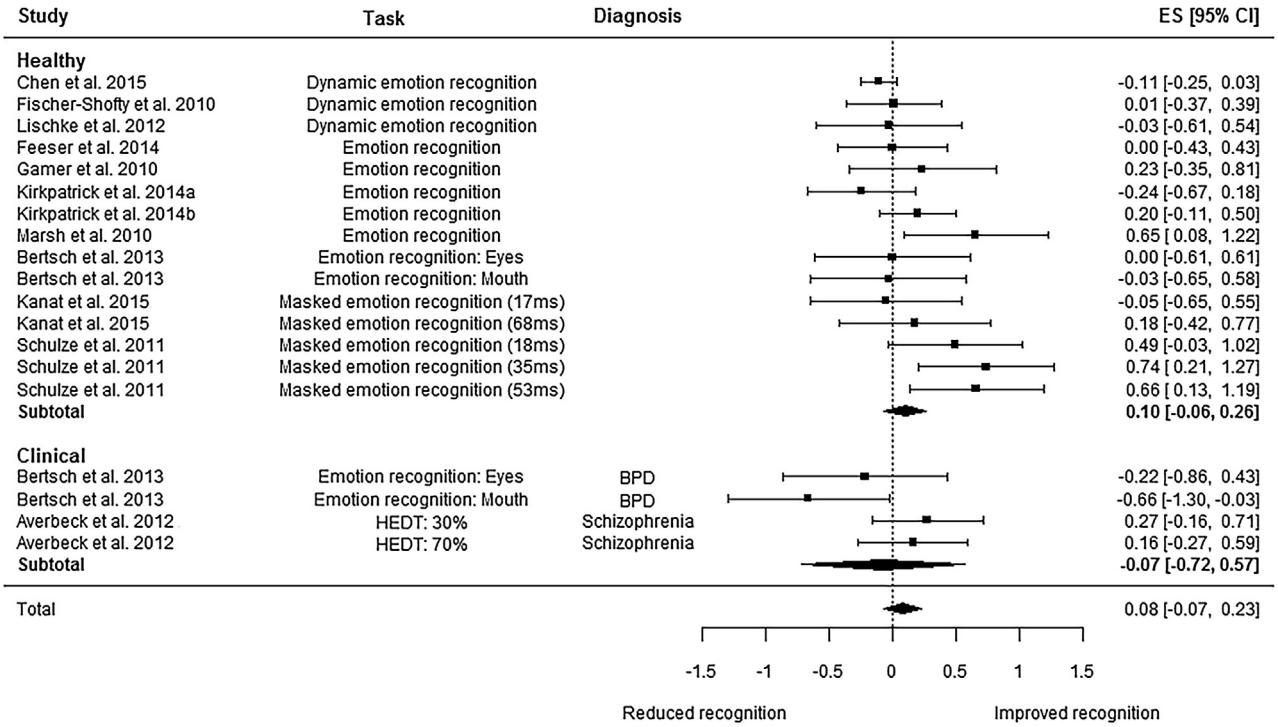

Fig. 10. Effect of oxytocin vs. placebo on recognition of happiness. Positive effect sizes indicate improved recognition of happiness following oxytocin administration; negative effect sizes indicate reduced recognition of happiness following oxytocin administration. HEDT: 30% = Hexagon emotion discrimination task: morphed faces 30% intensity; HEDT: 70% = Hexagon emotion discrimination task: morphed faces 70% intensity; BPD = Borderline personality disorder. Kirkpatrick et al., 2014: a = 20IU of intranasal oxytocin, b = 40IU of intranasal oxytocin.

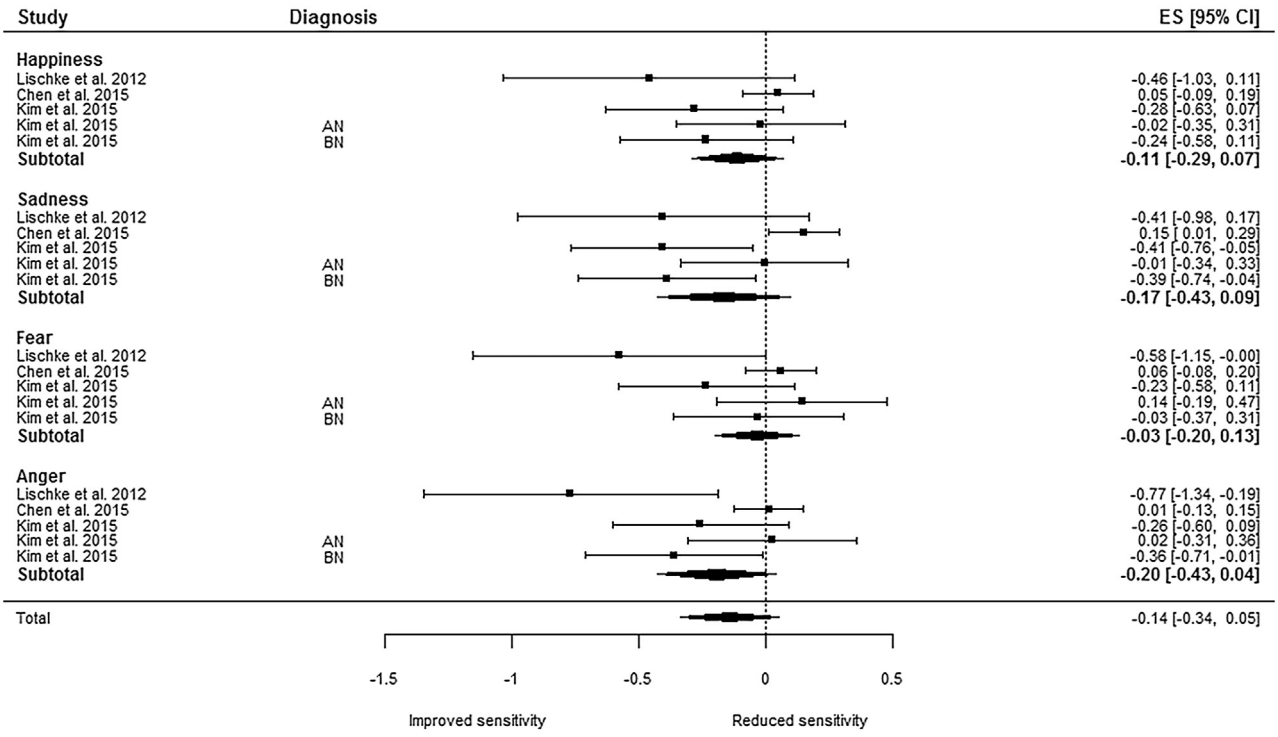

Fig. 11. Effect of oxytocin vs. placebo on emotion recognition sensitivity. Negative effect sizes indicate improved emotion recognition sensitivity following oxytocin administration; positive effect sizes indicate reduced emotion recognition sensitivity following oxytocin administration. All studies used dynamic emotion recognition task. BN = Bulimia nervosa, AN = Anorexia nervosa.

[-0.34, 0.05], k = 20, N of levels = 5). We then investigated recognition sensitivity of each emotion separately and found that oxytocin did not significantly improve the sensitivity to recognise happiness (ES = −0.11, Z = −1.24, p = 0.216, 95% CI [-0.29, 0.07], k = 5, N of levels = 5), sadness (ES = −0.17, Z = −1.25, p = 0.212, 95% CI [-0.43, 0.09], k = 5, N of levels = 5), fear (ES = −0.03, Z = −0.42, p = 0.674, 95% CI [-0.20, 0.13], k = 5, N of levels = 5), or anger (ES = −0.20, Z = −1.64, p = 0.100, 95% CI [-0.43, 0.04], k = 5, N of levels = 5).

The between-study heterogeneity approached significance (Q = 29.31, p = 0.061, I^2 = 67.31%) and was explored further with meta-regressions. The findings from the meta-regressions were corrected for multiple comparisons with Bonferroni correction (0.05/5) and p < 0.01 was considered significant. The proportion of female participants in the sample (Qm = 0.03, p = 0.864; Supplementary Fig. 23), age (Qm = 1.27, p = 0.261; Supplementary Fig. 24), the dose administered (Qm = 0.03, p = 0.864; Supplementary Fig. 25), and the diagnostic group (Qm = 2.03, p = 0.567) did not significantly explain the heterogeneity.

Begg's rank correlation test of funnel plot asymmetry revealed evidence of significant publication bias (T = −0.74, p < 0.0001; Supplementary Fig. 26) indicating that small studies finding larger effects were more likely to be published.

3.3. Effects of oxytocin on emotion expression

Four studies investigated the effects of a single dose of intranasal oxytocin on expression of congruent emotions in response to emotionally provoking stimuli (Fig. 12). Three studies included individuals with clinical conditions, including BPD, Schizophrenia, and AN.

Overall, there was no significant effect of oxytocin on emotion expression (ES = 0.08, Z = 0.87, p = 0.385, 95% CI [-0.10, 0.26], k = 14, N of levels = 7). When the data was further inspected, the meta-analysis showed that oxytocin significantly increased the expression of positive emotions among the healthy individuals with a small effect size (ES = 0.25, Z = 2.29, p = 0.022, 95% CI [0.04, 0.47], k = 4, N of levels = 4). Oxytocin did not significantly influence the expression of negative emotions among the healthy individuals (ES = 0.10, Z = 0.55, p = 0.585, 95% CI [−0.27, 0.47], k = 4, N of levels = 4). There was also no evidence of significant effects of oxytocin on the expression of positive or negative emotions among the clinical populations (Positive ES = 0.02, Z = 0.13, p = 0.896, 95% CI [−0.22, 0.25], k = 3, N of levels = 3; Negative: ES = −0.08, Z −0.35, p = 0.726, 95% CI [−0.52, 0.36], k = 3, N of levels = 3).

There was significant between-study heterogeneity (Q = 23.14, p = 0.040, I^2 = 35.95%), which was explored further with meta-regressions. The proportion of female participants in the sample, age, and the dose administered individually significantly explained the heterogeneity (Supplementary Figs. 27–29). These variables were, thus, entered into a full model which significantly explained the between-study heterogeneity (Qm = 8.80, p = 0.032) leaving no significant residual heterogeneity (Qr = 14.33, p = 0.158). This finding suggests that older male participants who received 24IU of intranasal oxytocin showed greater oxytocin induced increase in facial expressivity. However, this meta-analysis consisted of only four studies and, thus, this finding should be interpreted with caution. The diagnostic group (Qm = 6.14, p = 0.105)did not significantly explain the heterogeneity.

There was no evidence of significant publication bias on the Begg's rank correlation test of funnel plot asymmetry (T = −0.03, p = 0.915; Supplementary Fig. 30). However, Rosenthal's file drawer analysis revealed a fail-safe N of 1, indicating that only 1 study finding no significant effects of oxytocin on emotion expression would be needed to reduce the observed significant effect to null. This does not exceed the Rosenthal's criterion for this meta-analysis ($5k+10 = 30$) indicating that this finding is not robust and there is likely to be substantial publication bias present.

4. Discussion

The aim of the current meta-analytic review was to investigate the

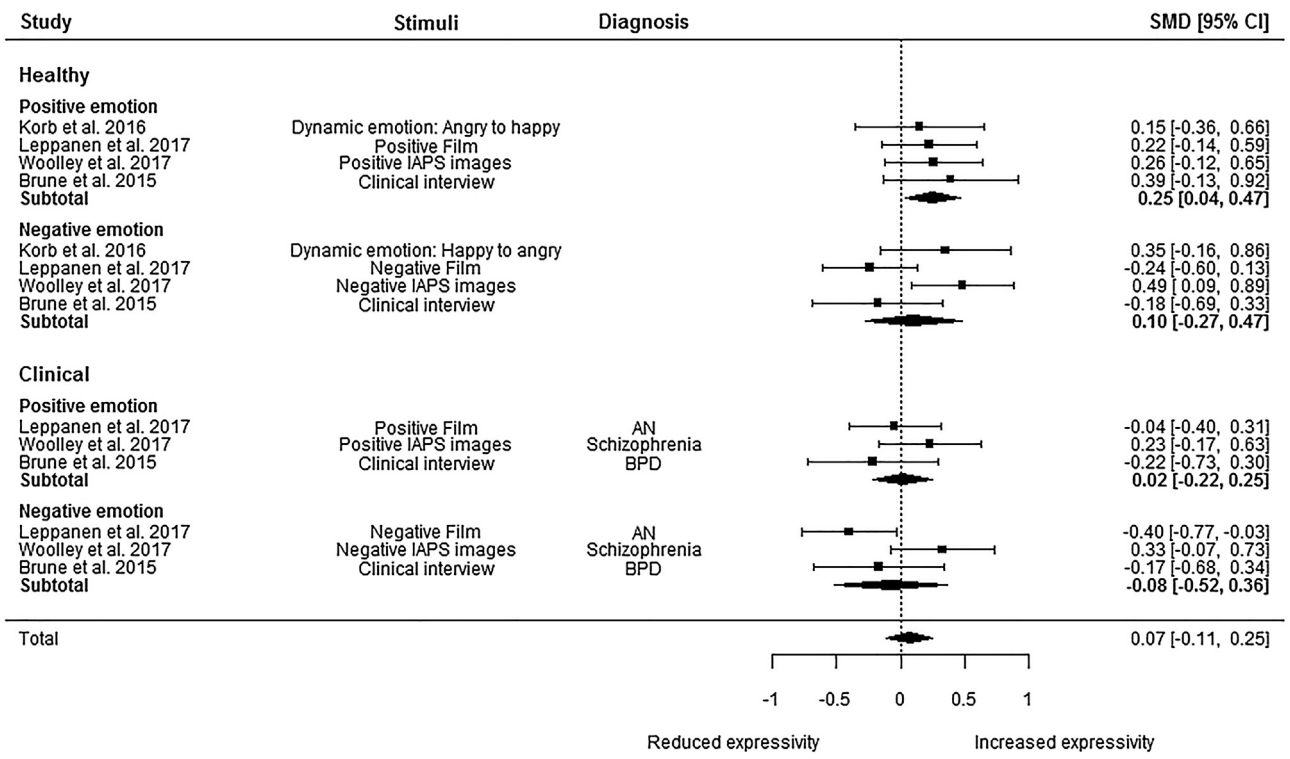

Fig. 12. Effect of oxytocin vs. placebo on emotion expression. Positive effect sizes indicate increased emotion expression following oxytocin administration; negative effect sizes indicate reduced emotion expression following oxytocin administration. IAPS = International affective picture system; AN = anorexia nervosa; BPD = Borderline personality disorder.

effects of a single dose of intranasal oxytocin on interpretation and expression of emotions among healthy and clinical populations. Most the studies recruited only healthy individuals, but thirteen studies also included people with clinical disorders and two studies included people with substance dependence disorder. The meta-analyses revealed that a single dose of intranasal oxytocin significantly improved the recognition of basic emotions, particularly fear, but only among healthy individuals with small to negligible effect sizes. Oxytocin also increased the expression of positive emotions with a small effect size among the healthy individuals. Intranasal oxytocin did not significantly influence theory of mind among the healthy individuals. Although, the oxytocin-induced improvement in basic emotion recognition approached significance, overall there were no significant effects on intranasal oxytocin on the interpretation or expression of emotions among the mixed clinical population.

The oxytocin-induced increase in the expression of positive emotions in healthy individuals is in line with findings from previous systematic reviews and suggests that oxytocin plays an important role in facilitating prosocial behaviour in humans (Bakermans-Kranenburg and van Ijzendoorn, 2013; Churchland and Winkielman, 2012; Guastella and MacLeod, 2012). This finding is also supported by previous work that has documented increased trust and cooperation particularly towards the members of a safe “in-group” following oxytocin administration (van Ijzendoorn and Bakermans-Kranenburg, 2012). Together these findings suggest that oxytocin may facilitate prosocial behaviour among healthy individuals. However, it is of importance to note that the oxytocin-induced increase in the expression of positive emotions was not robust, and it is likely that there was substantial publication bias present.

The present findings also showed that intranasal oxytocin improved recognition of basic emotions, which appeared to be largely driven by oxytocin-induced significant improvement in fear recognition and nearly significant improvement in disgust recognition. These results may seem surprising in this light of the findings above. However, these results are in line with a previous meta-analysis, which also found that intranasal oxytocin improved early recognition of angry faces and late recognition of fearful faces among healthy individuals (Shahrestani et al., 2013). Additionally, intranasal oxytocin has also been found to increase attention and approach towards negative social-emotional stimuli, such as angry and fearful facial expressions (Clark-Elford et al., 2015; Simon et al., 2012; Tollenaar et al., 2013).

Recent systematic reviews have attempted to explain the recent emergence of similar seemingly contradictory findings by suggesting that the effects of intranasal oxytocin may be modulated by social boundaries (Olff et al., 2013; Zik and Roberts, 2015). This hypothesis suggests that oxytocin may be of evolutionary importance in social interactions, increasing pro-social behaviour towards “safe” stimuli and defensiveness towards “unsafe” stimuli (Olff et al., 2013; Zik and Roberts, 2015). Thus, when faced with positive social-emotional cues oxytocin may facilitate pro-social behaviour, trust, and cooperation. Conversely, when presented with unfamiliar or negative stimuli oxytocin administration may increase attention and alertness towards these potentially threatening, negative social-emotional cues. Although this hypothesis has not been previously linked to the effects of oxytocin on social-emotional processing, it is consistent with the present findings and is supported by several behavioural studies using investment and trust games. These studies have documented that intranasal oxytocin increases empathy towards “in-group” members as well as cooperation and compliance within the “in-group” (De Dreu and Kret, 2016; Ten Velden et al., 2017). Oxytocin also reduced cooperation with “out-group” members even when the “out-group” members are generous towards the participants (Daughters et al., 2017).

Although, the oxytocin-induced improvement in basic emotion recognition approached significance, overall the present series of meta-analyses found no significant oxytocin-induced changes in interpretation or expression of emotions among the mixed clinical population. One possible explanation is that the different disorders included in the mixed clinical group were too heterogeneous and that there were too few studies with the same disorder group to draw firm conclusions. Indeed, there was significant heterogeneity between the diagnostic groups in the present series of meta-analyses. However, there were also substantial differences in effect size estimates between studies that

included patient groups with the same diagnosis, suggesting that there may be individual differences within the diagnostic groups that modulate the effects of oxytocin.

Large-scale cohort studies have documented that clinical populations have inter-individual variability with substantial heterogeneity in types of comorbidity and aetiological risk (Lamers et al., 2010; Melartin et al., 2002; Sterling et al., 2008; Wessman et al., 2009). A few recent systematic reviews have also suggested that the effects of oxytocin on social-emotional functioning may be moderated by contextual and individual differences (Bartz et al., 2011; Olff et al., 2013). Factors such as attachment style and experience of early parental care have been found to moderate the effects of oxytocin; those scoring low on attachment avoidance and harsh parenting showed greater oxytocin-induced increase in social cooperation and positive response to crying infants (Bakermans-Kranenburg et al., 2011; Fang et al., 2014; Olff et al., 2013). Thus, further investigation of potential contextual and individual differences that may modulate the effects of oxytocin is of interest.

4.1. Limitations, recommendations and future directions

The majority of the studies included in the present review did not meet the sample size requirement for adequate statistical power to reliably detect at least a moderate effect of the drug. Although meta-analyses are a powerful method to pool studies and increase statistical power (Greco et al., 2013), it is important that individual studies also have adequate power. This has been suggested to be a particularly big problem in oxytocin research and the potential source of the wide range of different and sometimes contradictory findings arising from different studies (Walum et al., 2016). These problems may go some way to explain the between-study heterogeneity and publication bias in the present review. Therefore, we recommend that future studies should recruit much larger number of participants to ensure that reliable effects of intranasal oxytocin can be detected.

Several studies investigating the effects of a single dose of intranasal oxytocin on recognition of basic emotions had evidence of ceiling effects with mean accuracy percentages over 85%. Even though presence of ceiling effects did not have significant impact on the present findings, such effects make it difficult to find the true effect of the drug or intervention because performance is already at maximum. Future studies investigating the effects of intranasal oxytocin on the recognition of basic emotions, should opt for alternative tasks, for example presenting images where the emotion is less than 100% present.

Six of the meta-analyses in the present review had evidence of between-study heterogeneity and in four of them we were unable to identify the source of the heterogeneity. Previous studies have reported several confounding factors such as individual differences in anxiety and attachment security that can influence the effects of oxytocin on social-emotional processing (Bakermans-Kranenburg et al., 2011; Fang et al., 2014; Olff et al., 2013). The impact of these factors were not explored or reported in majority of the studies included in the present review meaning that it was not possible for us to explore the impact of these factors had on the meta-analyses. We recommend that future studies explore the impact of individual differences to gain better understanding of the impact of potential confounding factors on the effects of intranasal oxytocin.

Finally, there has recently been some doubt regarding the effects of intranasal oxytocin on social-emotional functioning in general and criticism directed at the lack of compelling theoretical framework to explain the contradictory findings reported thus far (Lane et al., 2016; Leng and Ludwig, 2016). Some important questions have also been raised regarding if and how intranasal oxytocin accesses the brain and in what quantities (Leng and Ludwig, 2016). These criticisms have become increasingly important to address in the light of recent replication failures (Lane et al., 2015; Radke and de Bruijn, 2015). We have taken some steps in the present review to introduce a potential theoretical framework, but several issues still remain unanswered. For instance, there are a number of methodological obstacles, such as individual differences in the nasal cavity physiology, the nasal spray formulations used, and the devices used to deliver the drug, that need to be considered (Quintana et al., 2016). Furthermore, there are uncertainties regarding the site and mechanism of action of exogenous intranasal oxytocin in humans due to lack of suitable radio tracers (Paloyelis et al., 2016). There has also been some suggestion that oxytocin has important effects in the periphery, moderating cardiovascular functioning and the peripheral cortisol response, which may influence social behaviour (Cardoso et al., 2014b; Gutkowska et al., 2014). Further research is needed to answer these important questions particularly if oxytocin is to be used as a treatment enhancer to support clinical care.

5. Conclusions

The current meta-analytic review pooled studies investigating the effects of a single dose of intranasal oxytocin on social-cognition. There was no significant effect of intranasal oxytocin on interpretation or expression of emotions among the mixed clinical population. Intranasal oxytocin significantly improved recognition of basic emotions, particularly fear, and increased the expression of positive emotions, but only among the healthy individuals. These findings are in line with previous work that has found that the effects of oxytocin may be modulated by social boundaries. Further large-scale research is needed to better understand of the role of intranasal oxytocin in social-emotional processing and potential moderators of its' effects.

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The authors do not report any financial or non-financial conflicting interests.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neubiorev.2017.04.010. The data and R commands used are available in the Open Science Framework (url: https://osf.io/v7nzb/).

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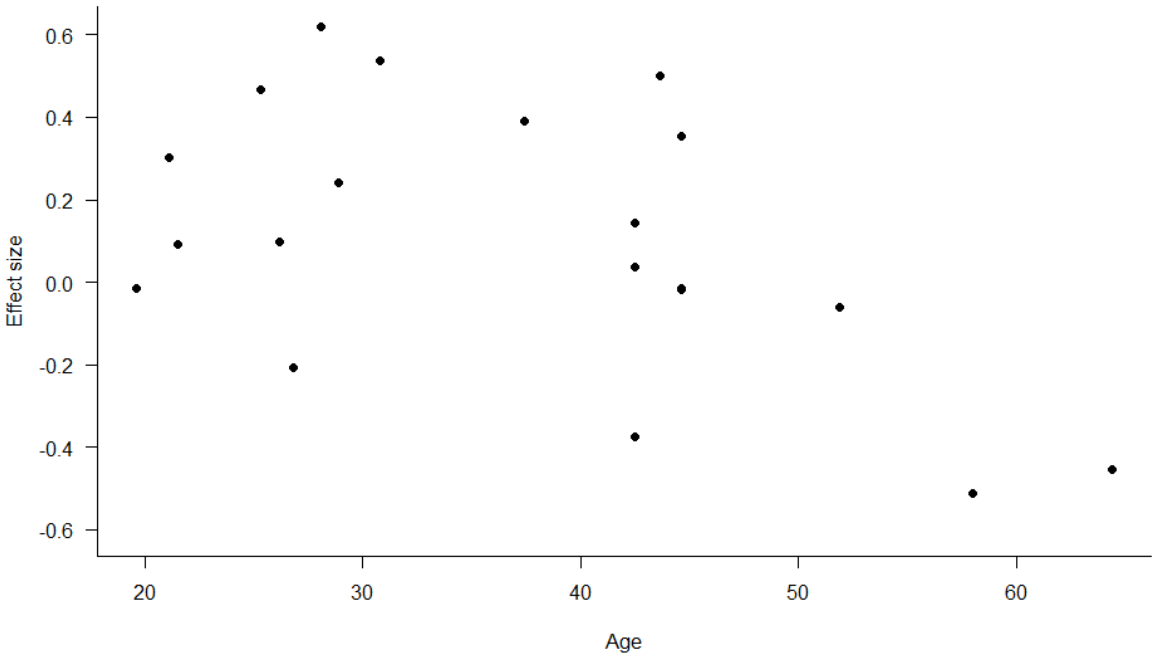
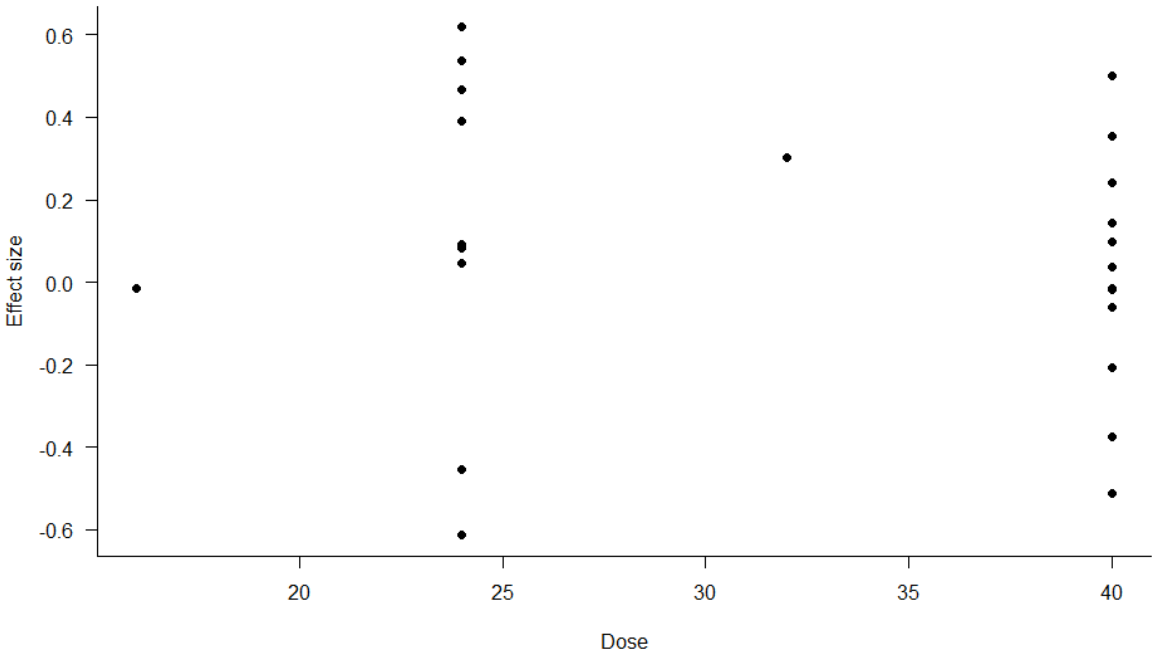
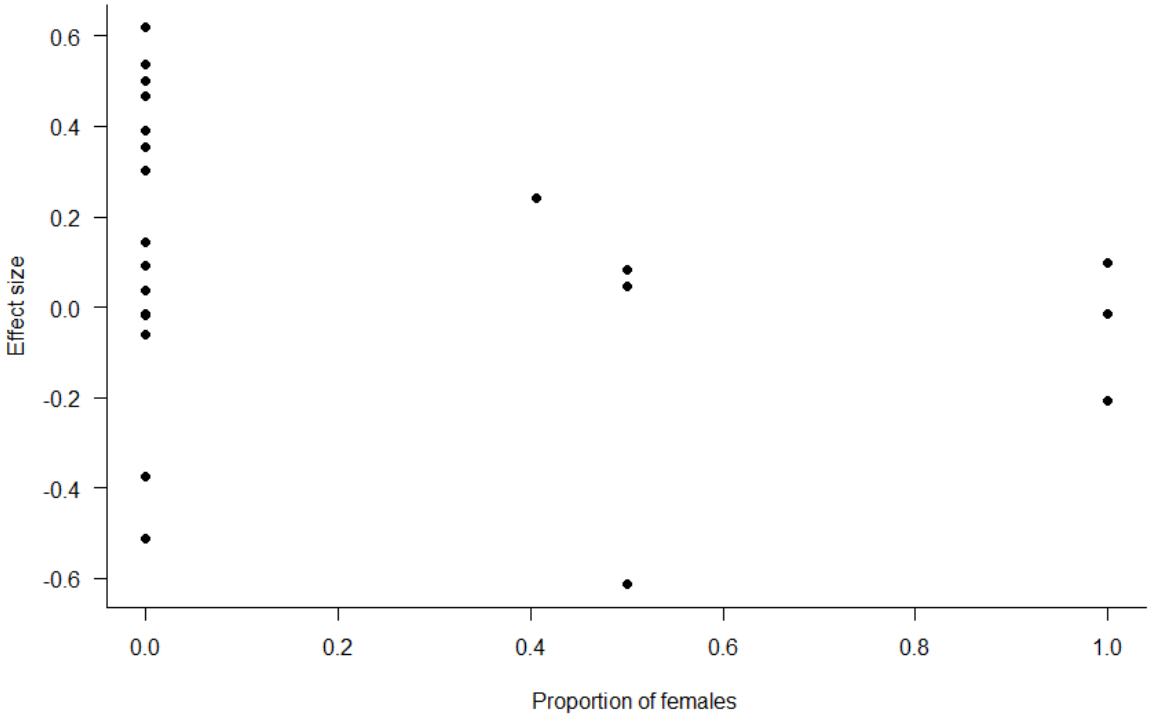
Supplementary Figure 1. Scatterplot of the association between age and effect size estimates in the meta-analysis investigating effects of a single dose of intranasal oxytocin on theory of mind.



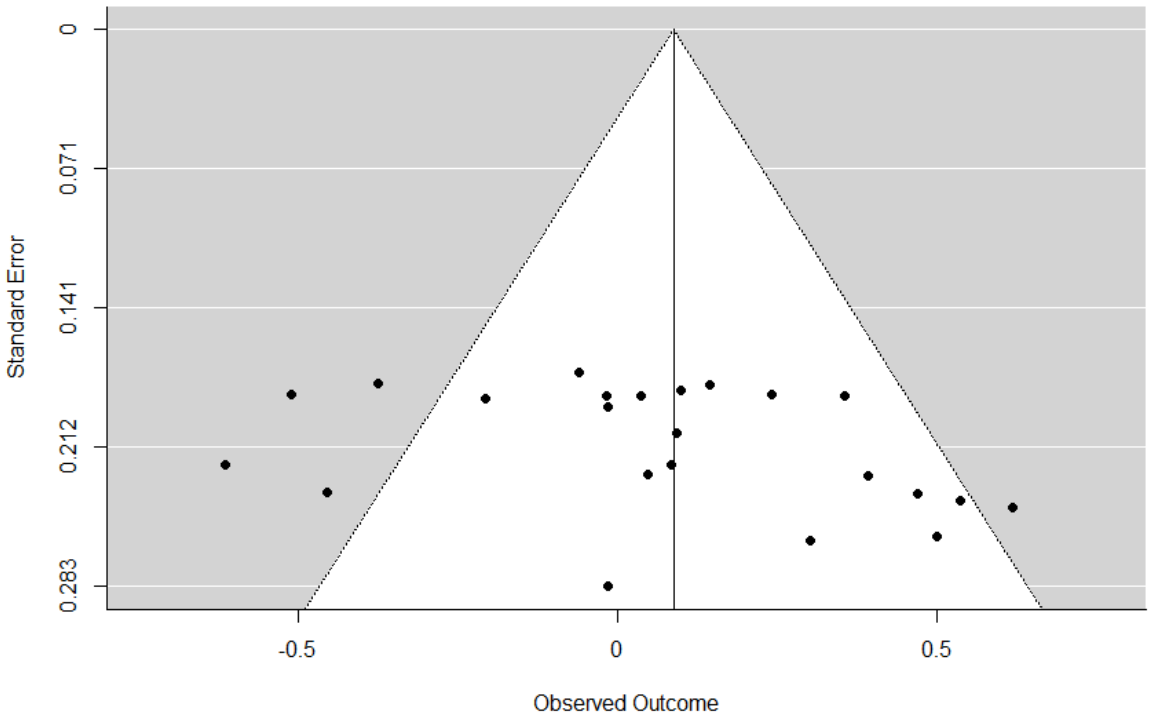
Supplementary Figure 2. Scatterplot of the association between dose (in IU) and effect size estimates in the meta-analysis investigating effects of a single dose of intranasal oxytocin on theory of mind.



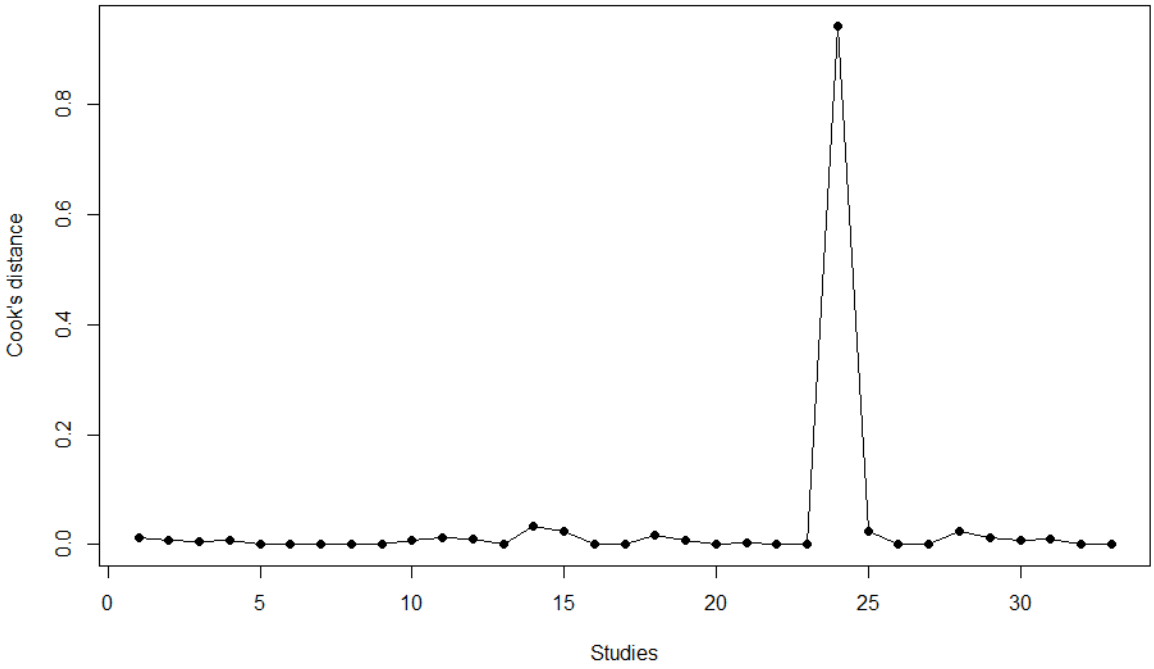
Supplementary Figure 3. Scatterplot of the association between proportion of female participants in the sample and effect size estimates in the meta-analysis investigating effects of a single dose of intranasal oxytocin on theory of mind.



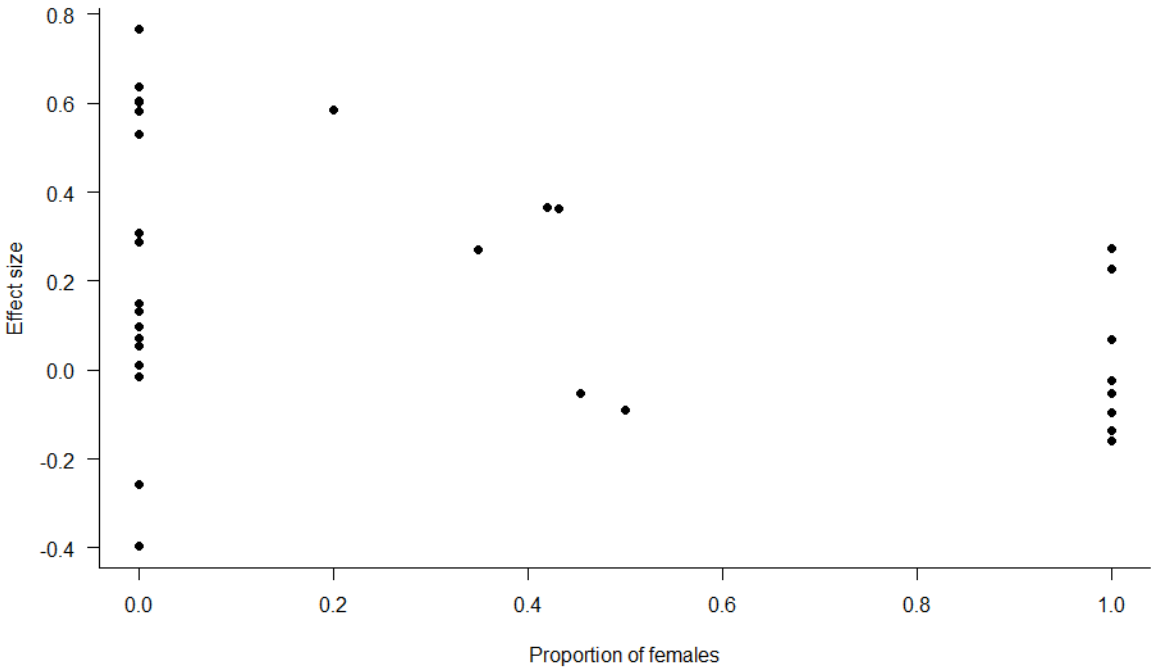
Supplementary Figure 4. Funnel plot of the meta-analysis investigating effects of a single dose of intranasal oxytocin on theory of mind.



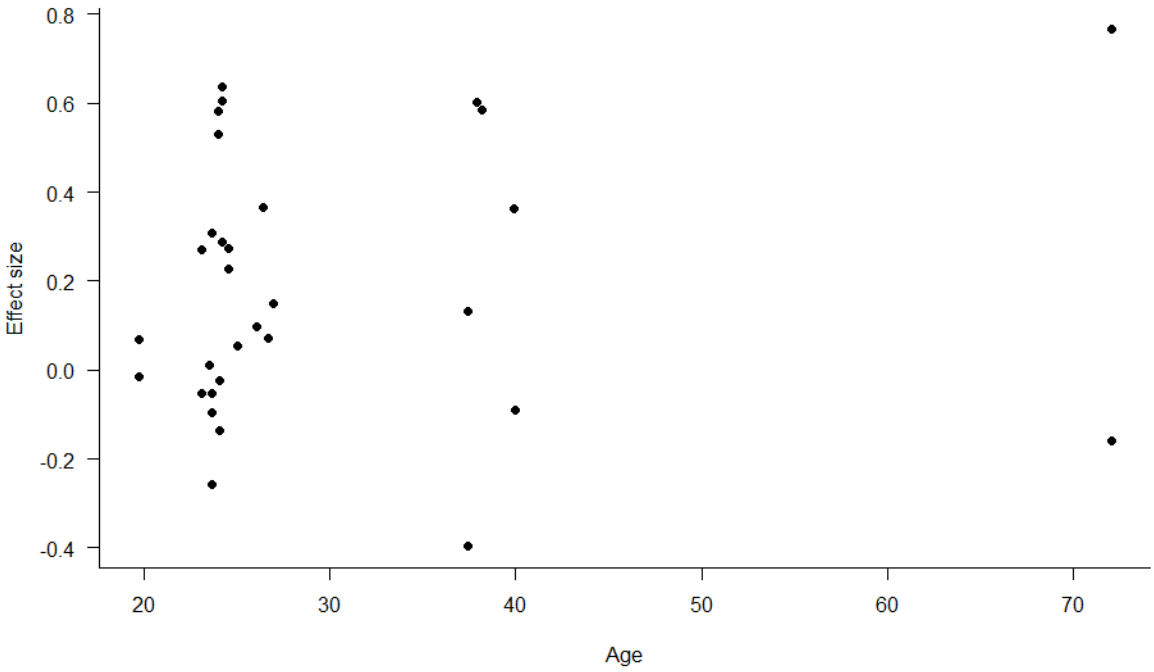
Supplementary Figure 5. Cook's Distance plot of all studies initially included in the meta-analysis investigating the effects of a single dose of oxytocin on basic emotions recognition. The black dots represent individual studies.



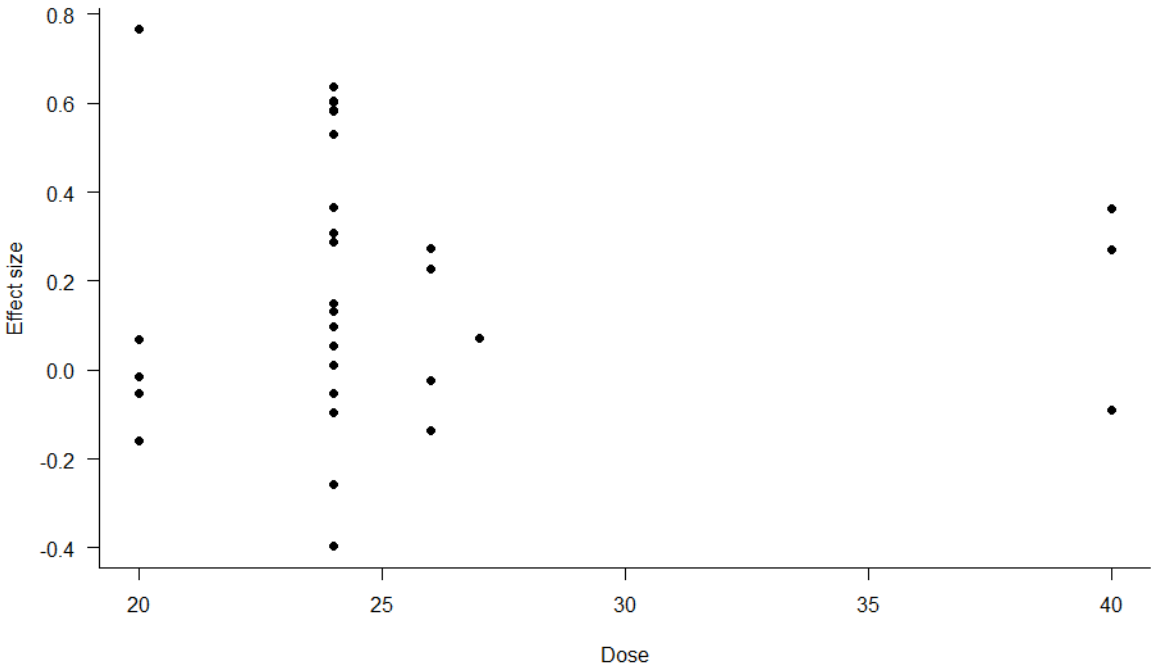
Supplementary Figure 6. Scatterplot of the association between proportion of female participants in the sample and effect size estimates in the meta-analysis investigating the effects of a single dose of oxytocin on basic emotions recognition.



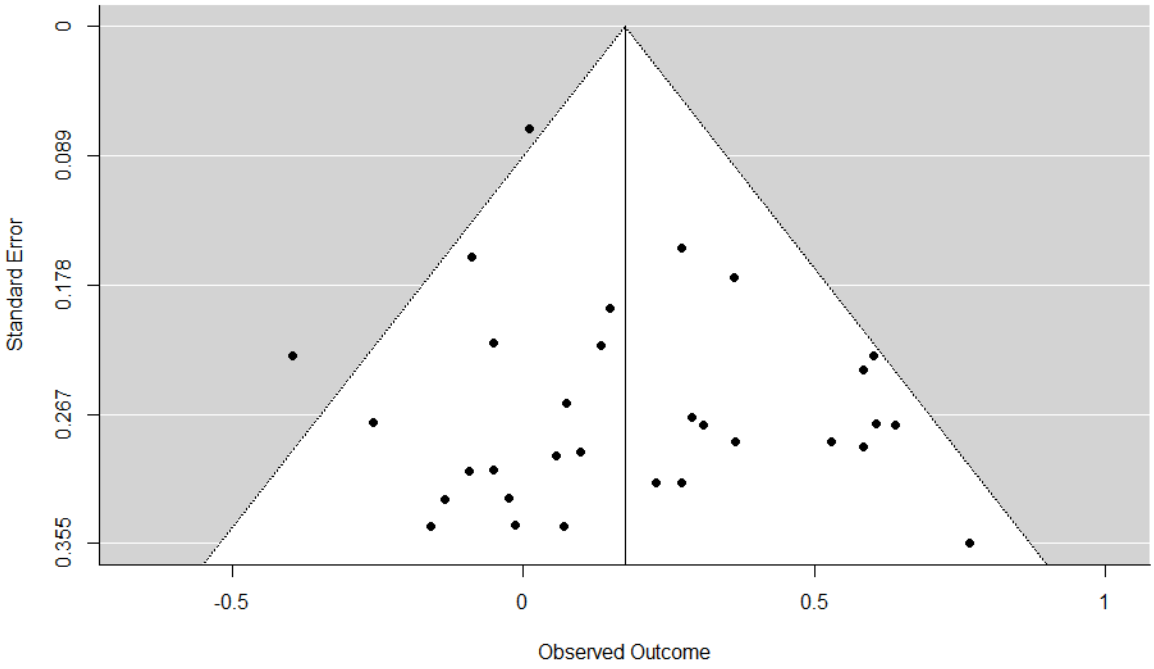
Supplementary Figure 7. Scatterplot of the association between age and effect size estimates in the meta-analysis investigating the effects of a single dose of oxytocin on basic emotions recognition.



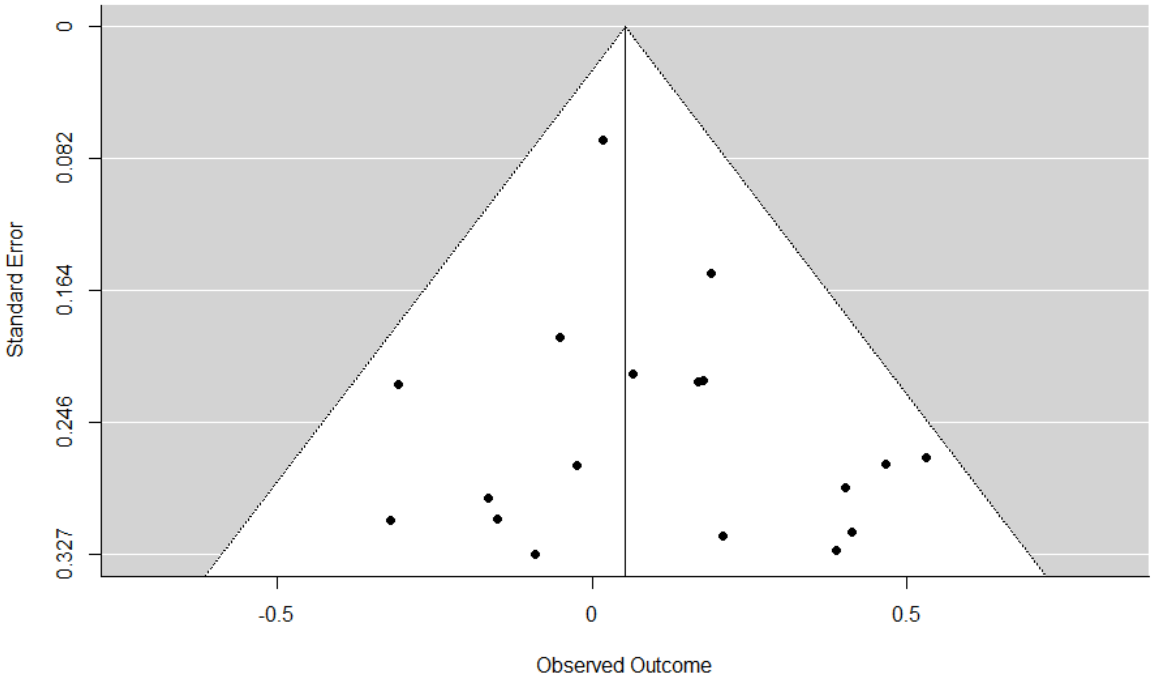
Supplementary Figure 8. Scatterplot of the association between dose (in IU) and effect size estimates in the meta-analysis investigating the effects of a single dose of oxytocin on basic emotions recognition.



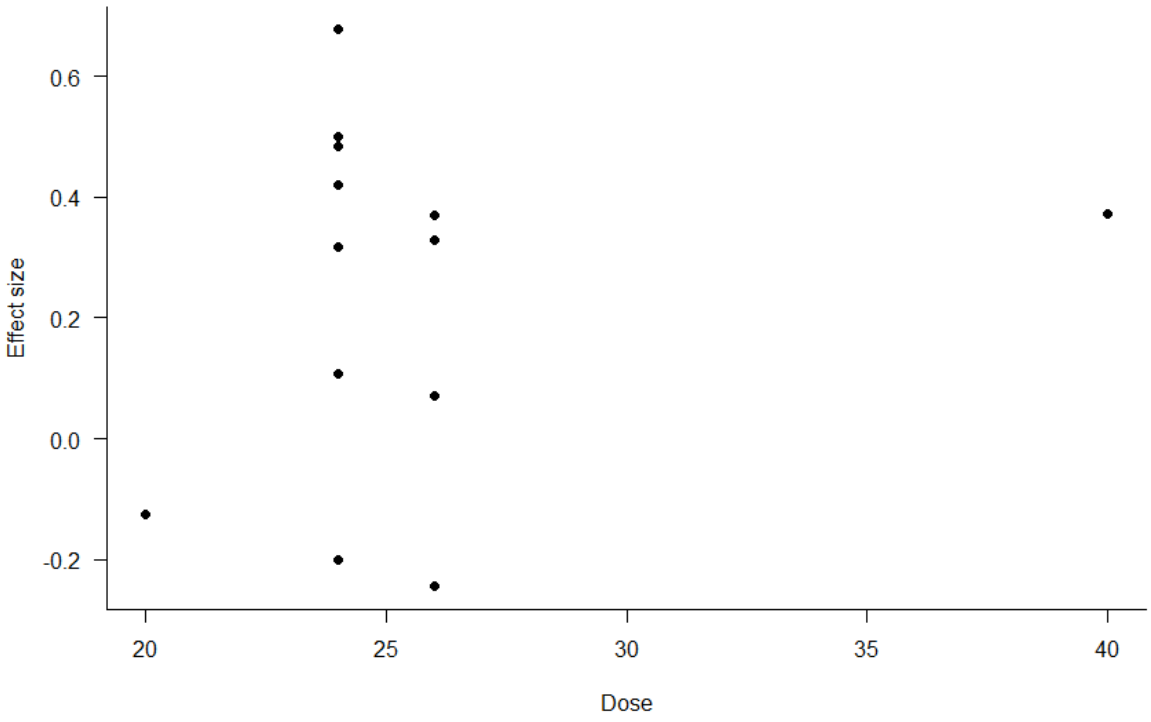
Supplementary Figure 9. Funnel plot of the meta-analysis investigating effects of a single dose of intranasal oxytocin on basic emotions recognition.



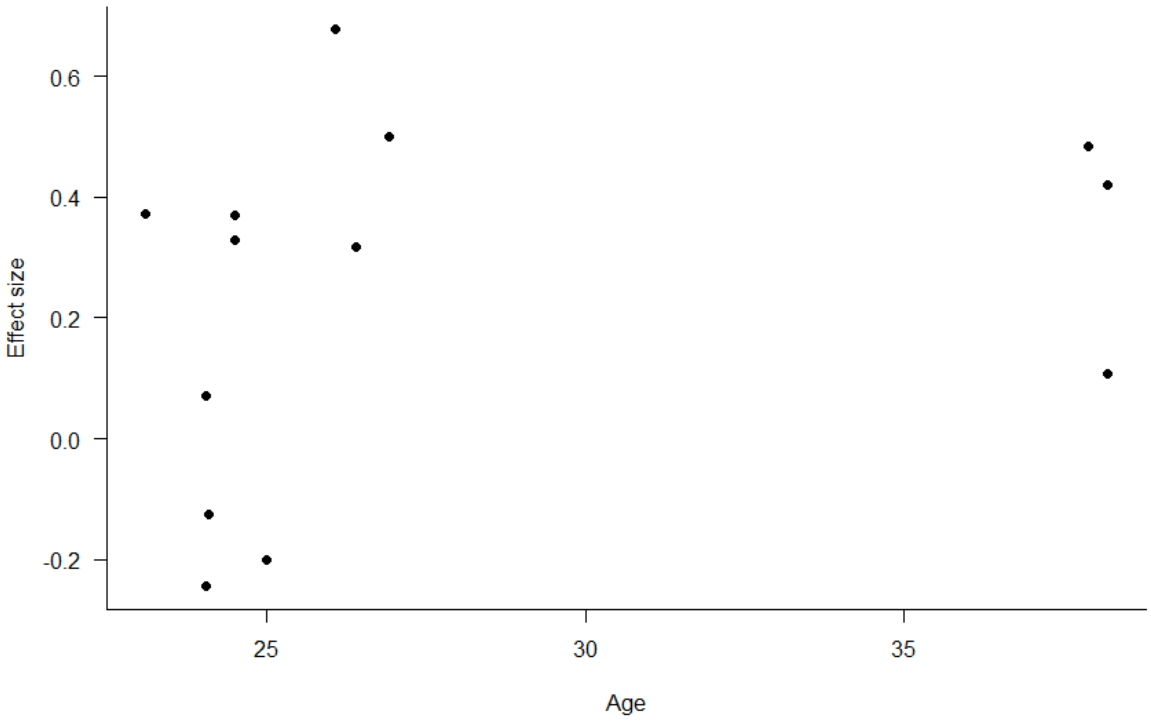
Supplementary Figure 10. Funnel plot of the meta-analysis investigating effects of a single dose of intranasal oxytocin on recognition of anger.



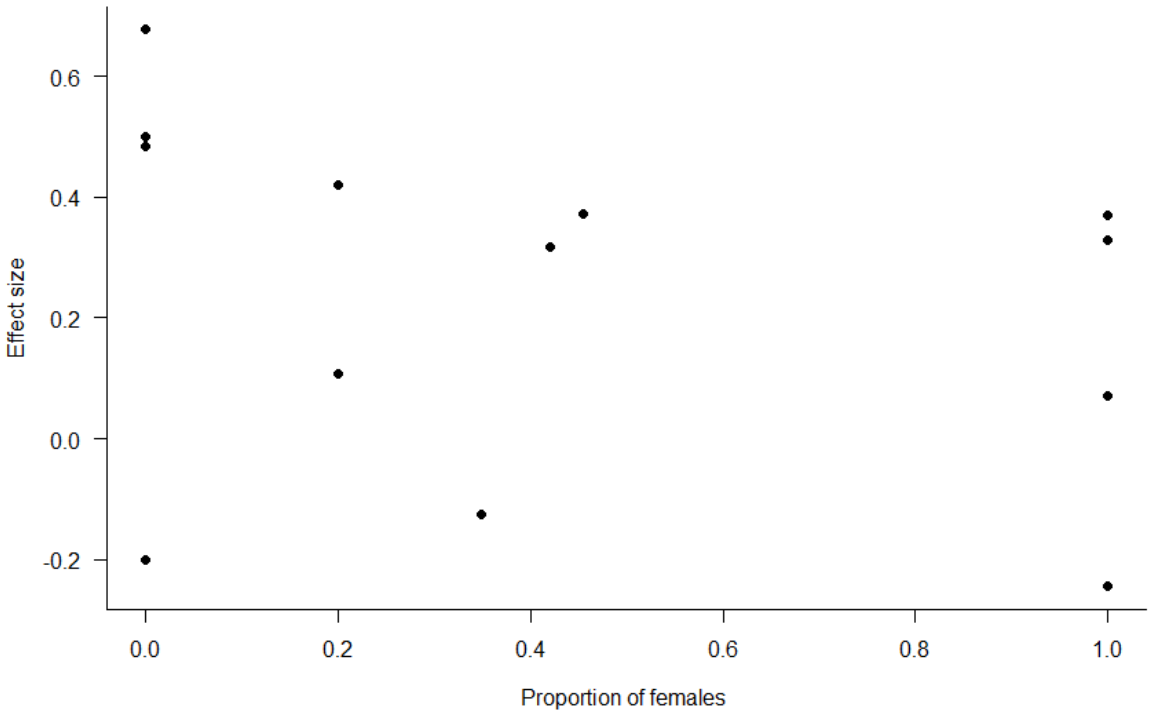
Supplementary Figure 11. Scatterplot of the association between dose (in IU) and effect size estimates in the meta-analysis investigating the effects of a single dose of oxytocin on recognition of fear.



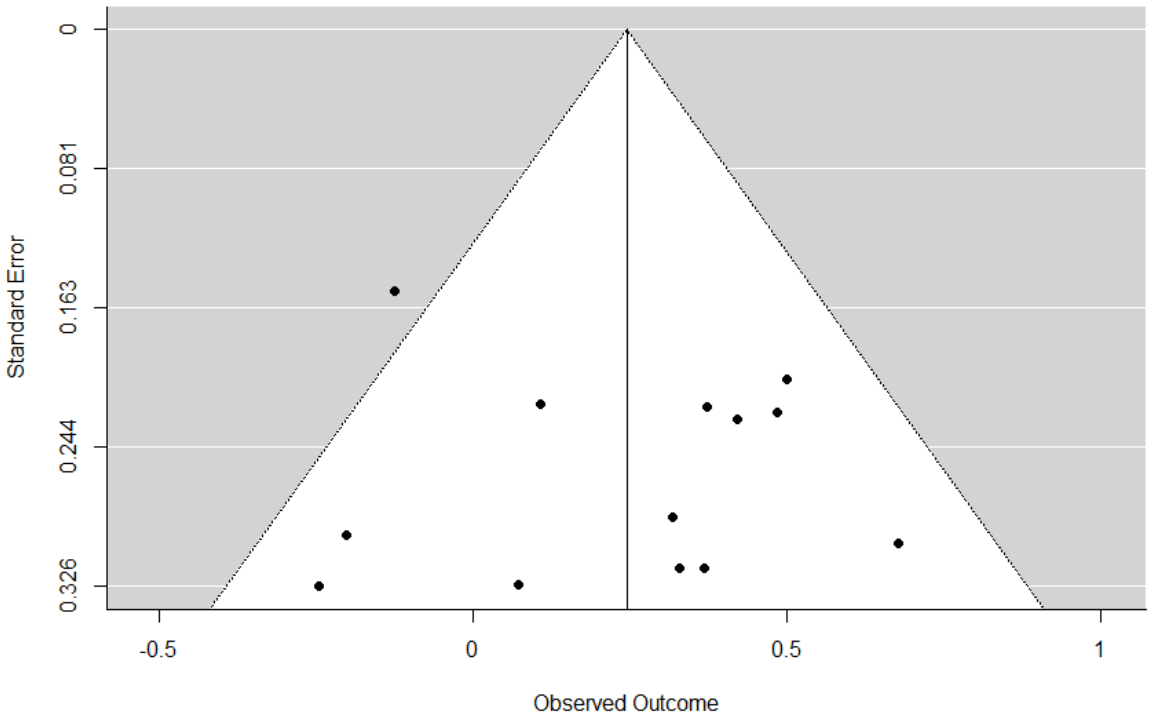
Supplementary Figure 12. Scatterplot of the association between age and effect size estimates in the meta-analysis investigating the effects of a single dose of oxytocin on recognition of fear.



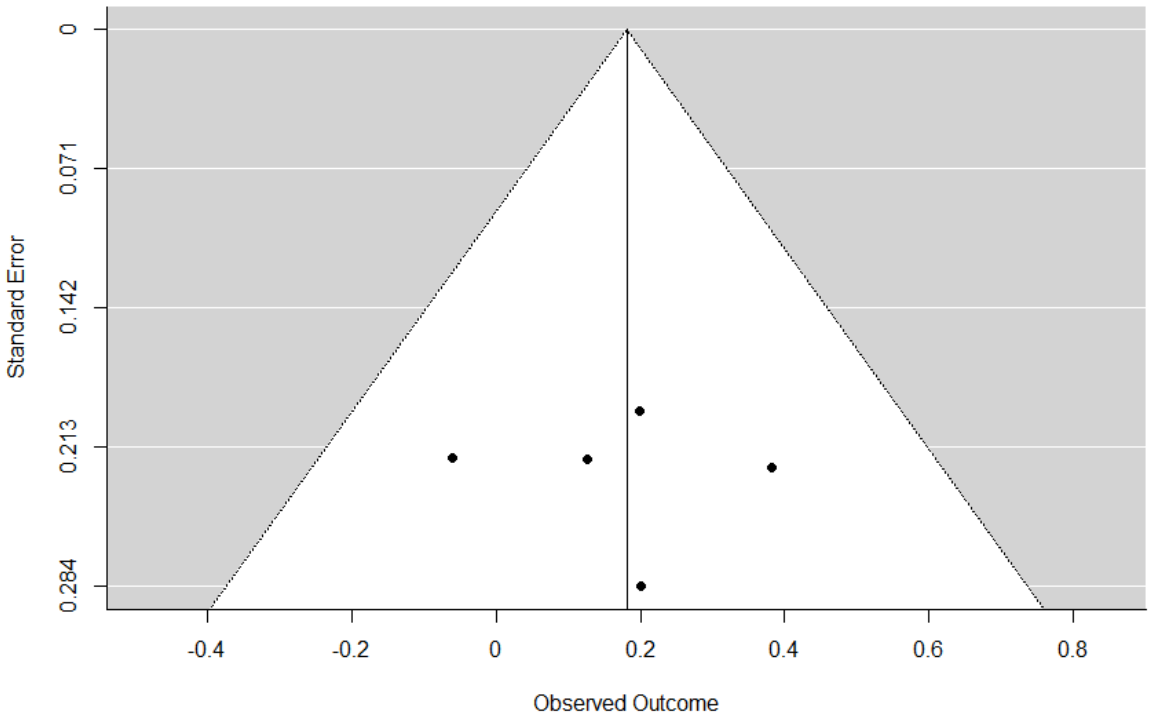
Supplementary Figure 13. Scatterplot of the association between proportion of female participants in the sample and effect size estimates in the meta-analysis investigating the effects of a single dose of oxytocin on recognition of fear.



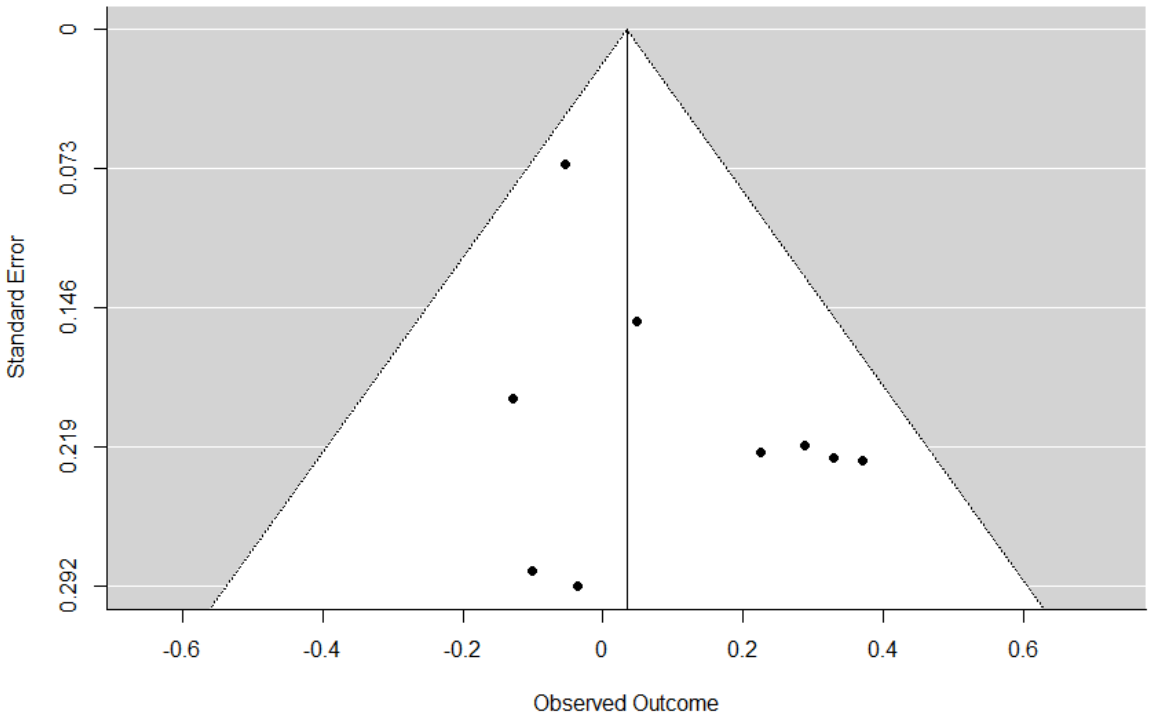
Supplementary Figure 14. Funnel plot of the meta-analysis investigating effects of a single dose of intranasal oxytocin on recognition of fear.



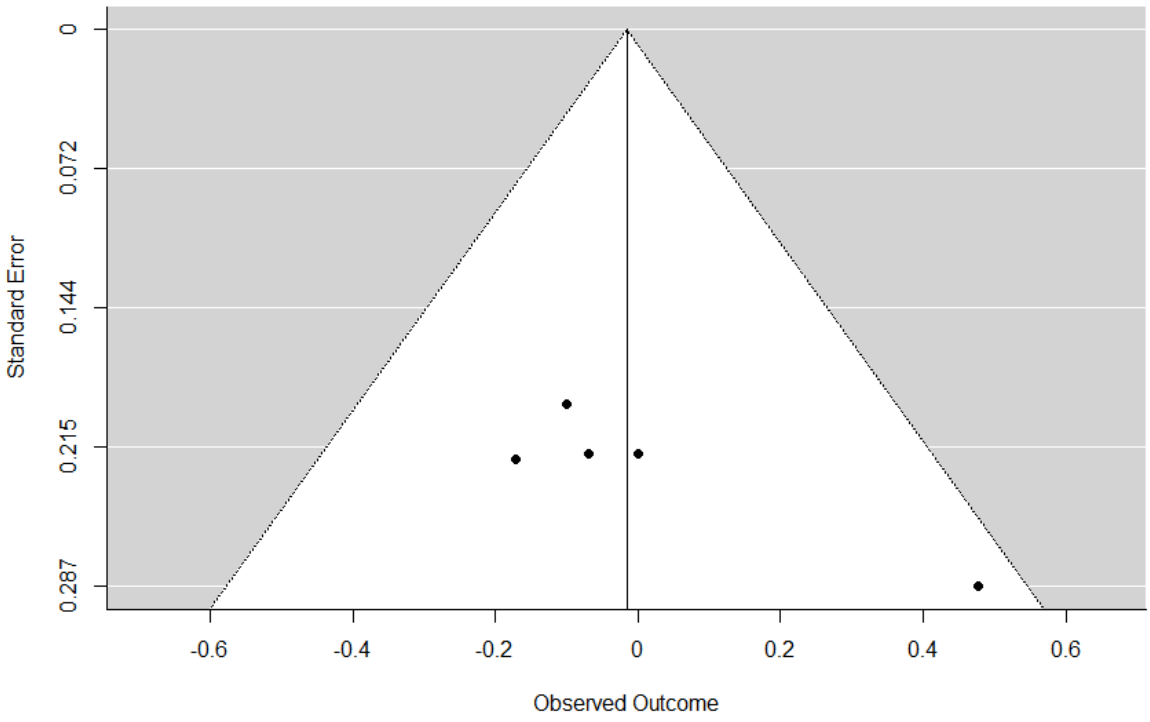
Supplementary Figure 15. Funnel plot of the meta-analysis investigating effects of a single dose of intranasal oxytocin on recognition of disgust.



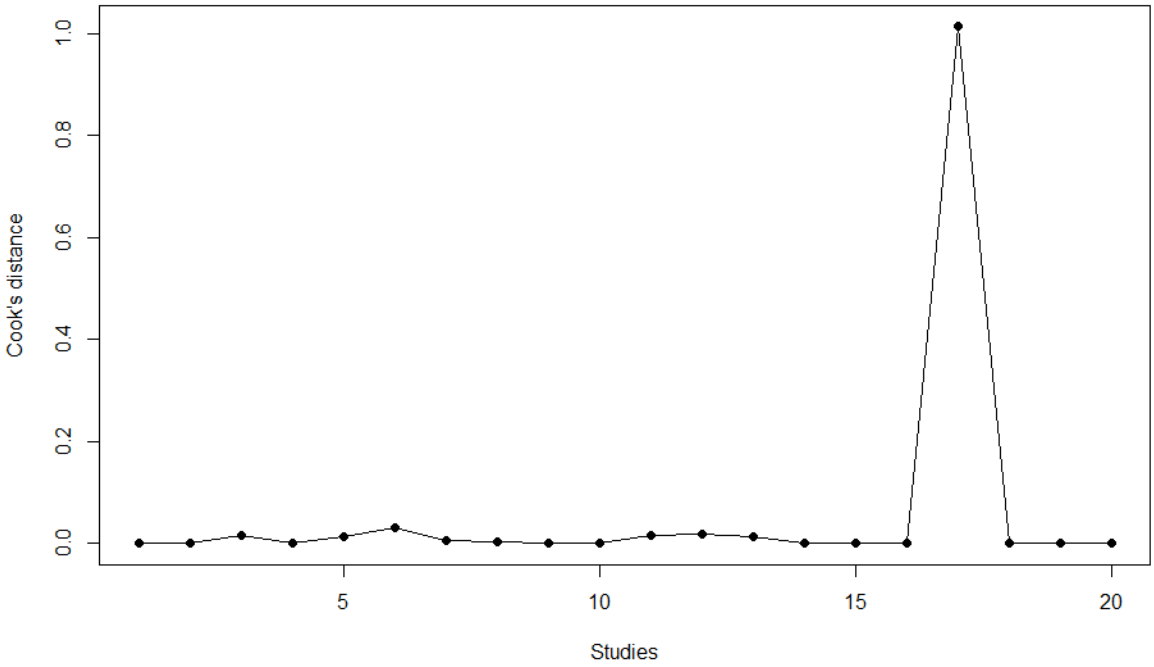
Supplementary Figure 16. Funnel plot of the meta-analysis investigating effects of a single dose of intranasal oxytocin on recognition of sadness.



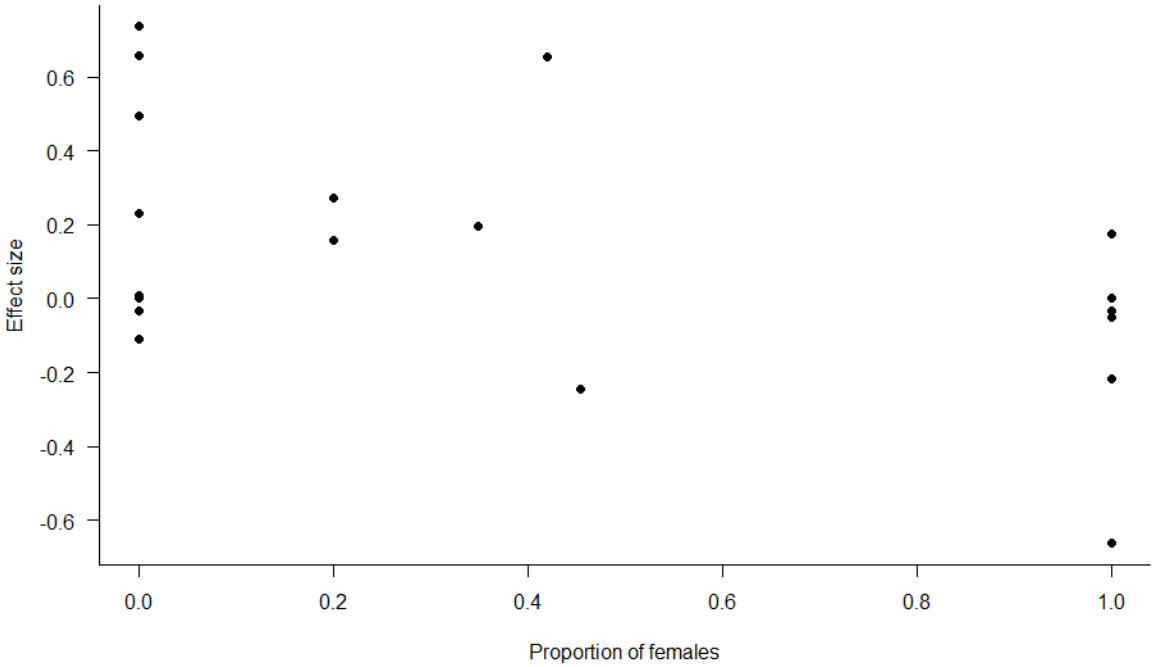
Supplementary Figure 17. Funnel plot of the meta-analysis investigating effects of a single dose of intranasal oxytocin on recognition of surprise.



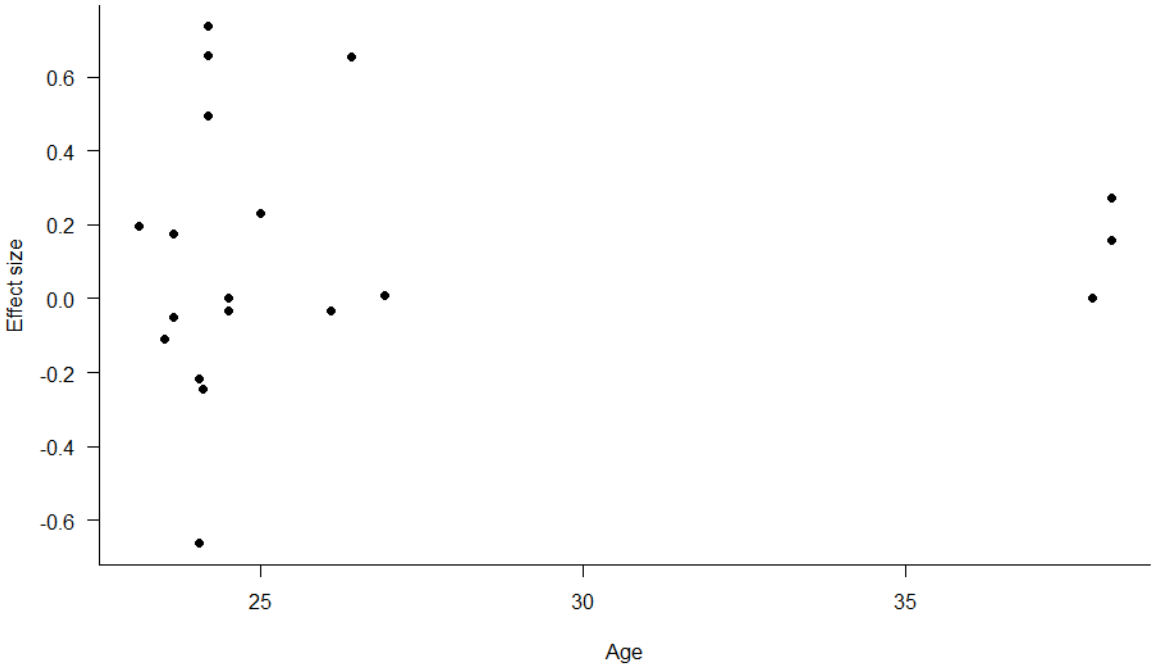
Supplementary Figure 18. Cook's Distance plot of all studies initially included in the meta-analysis investigating the effects of a single dose of oxytocin on recognition of happiness. The black dots represent individual studies.



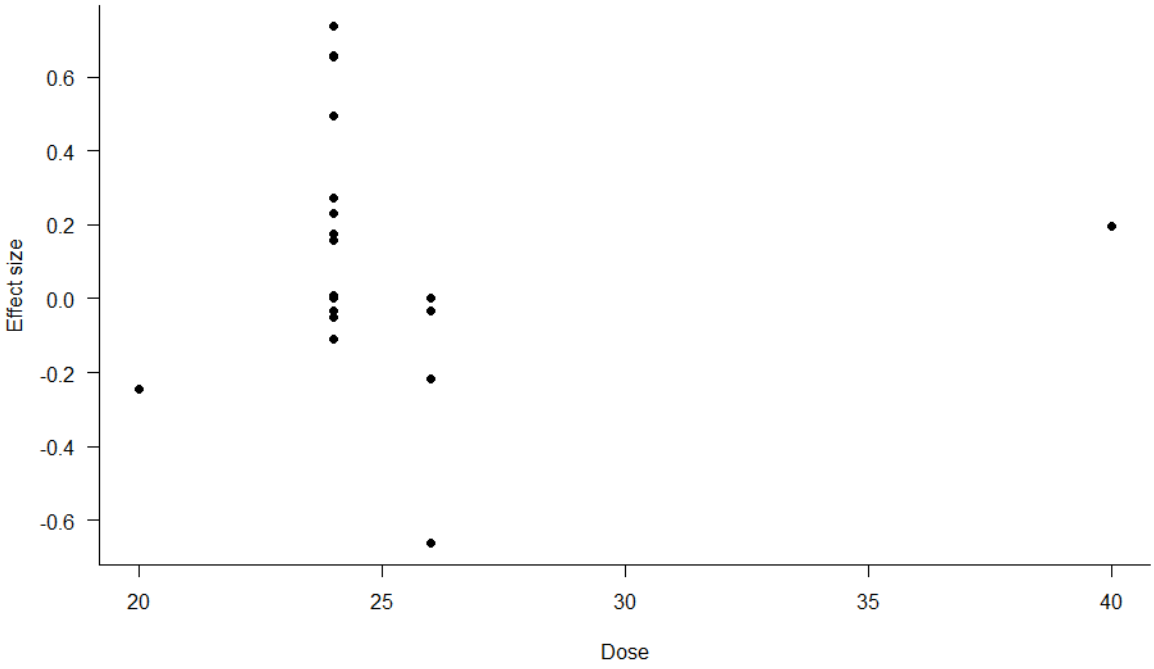
Supplementary Figure 19. Scatterplot of the association between proportion of female participants in the sample and effect size estimates in the meta-analysis investigating effects of a single dose of intranasal oxytocin on recognition of happiness.



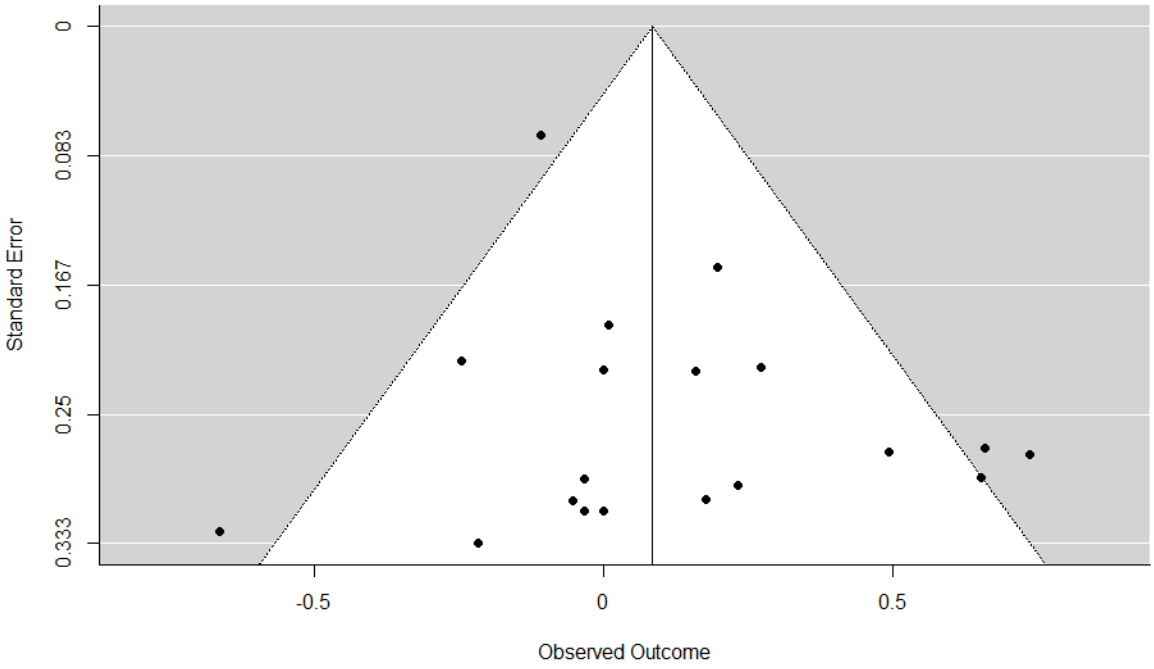
Supplementary Figure 20. Scatterplot of the association between age and effect size estimates in the meta-analysis investigating effects of a single dose of intranasal oxytocin on recognition of happiness.



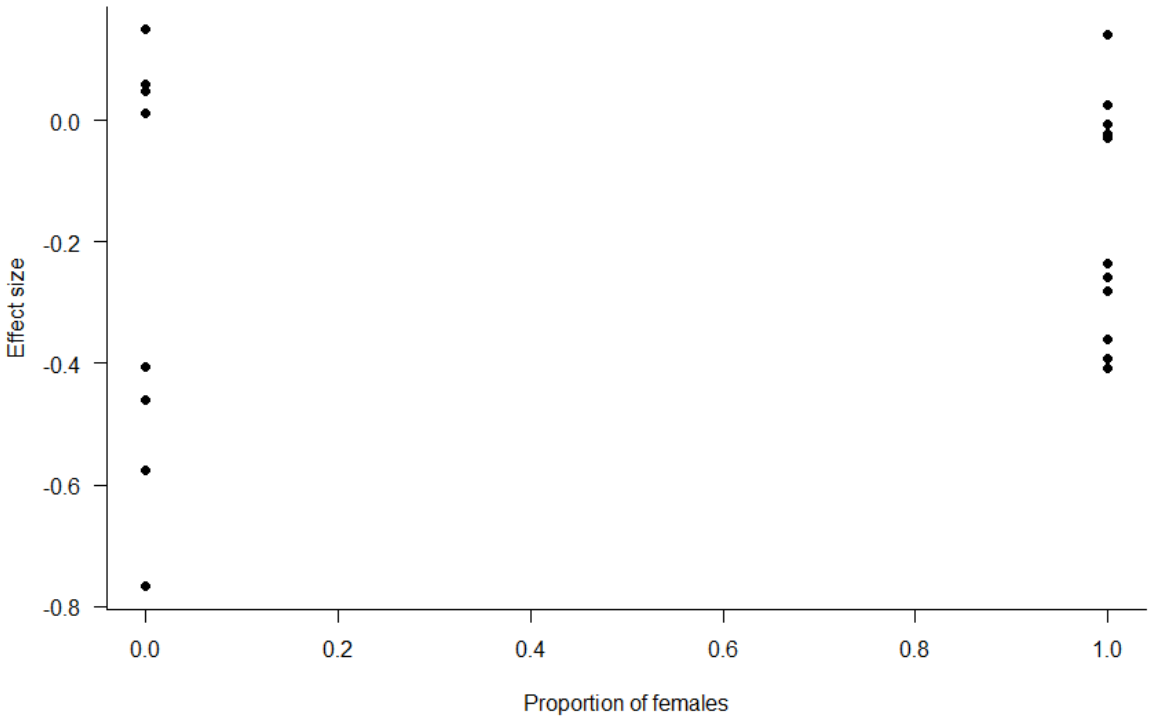
Supplementary Figure 21. Scatterplot of the association between dose (in IU) and effect size estimates in the meta-analysis investigating effects of a single dose of intranasal oxytocin on recognition of happiness.



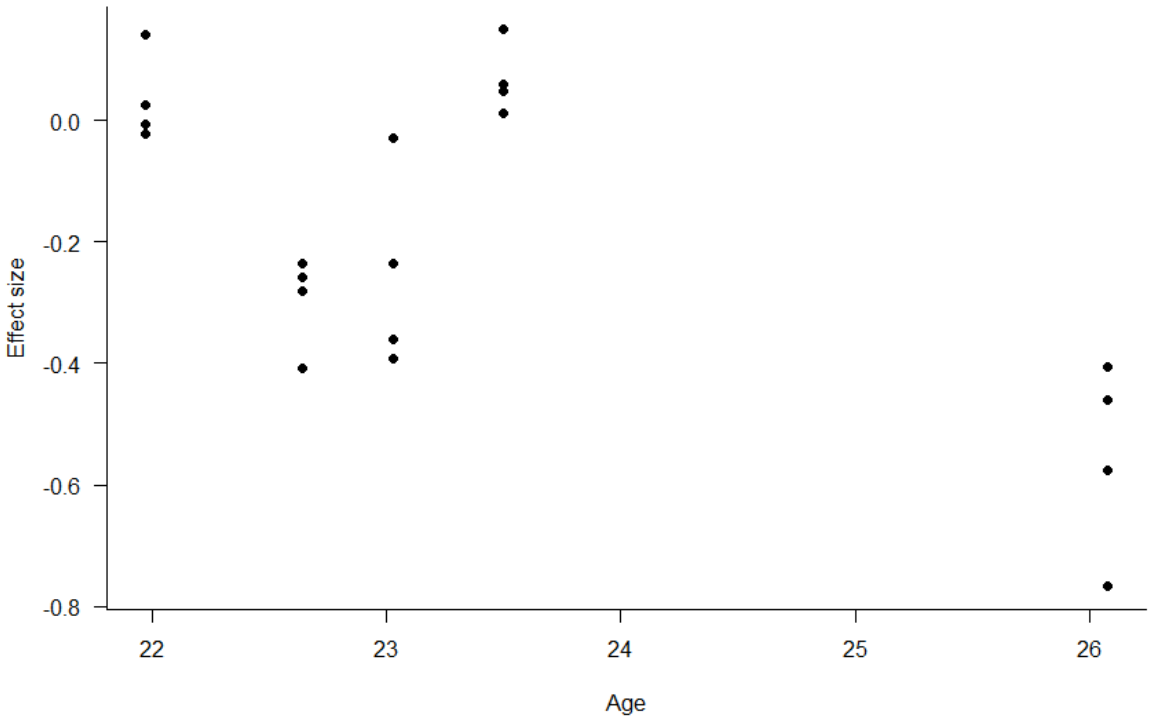
Supplementary Figure 22. Funnel plot of the meta-analysis investigating effects of a single dose of intranasal oxytocin on recognition of happiness.



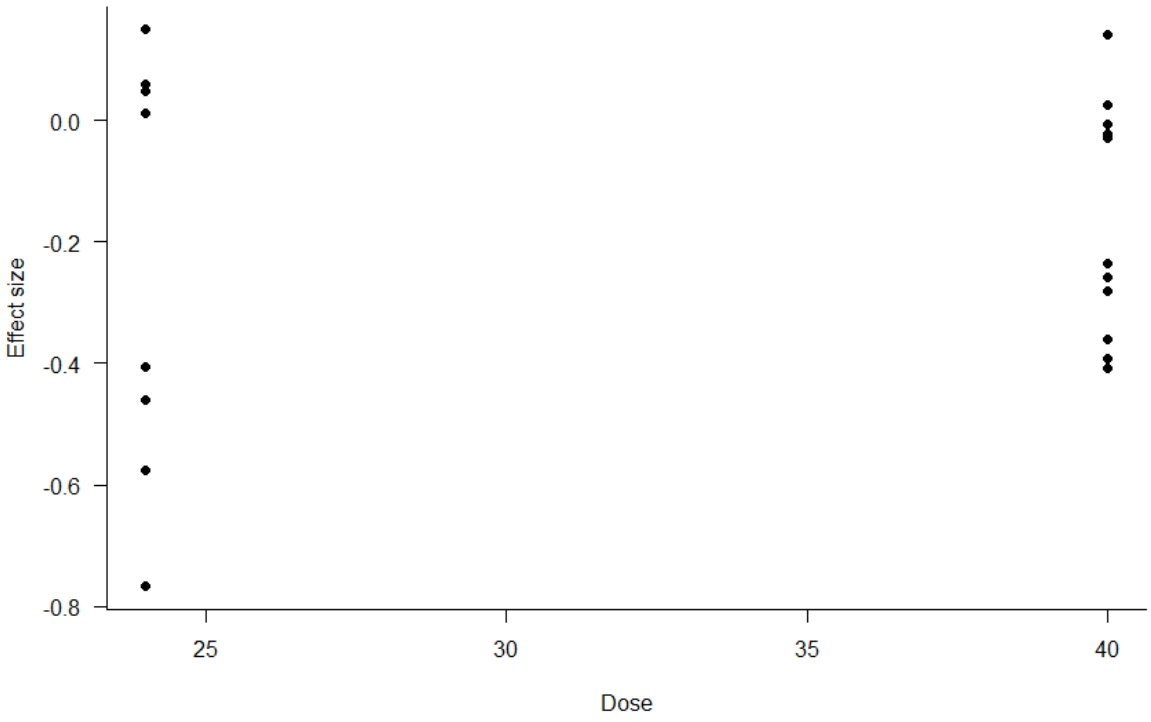
Supplementary Figure 23. Scatterplot of the association between proportion of female participants in the sample and effect size estimates in the meta-analysis investigating effects of a single dose of intranasal oxytocin on sensitivity to recognise emotions.



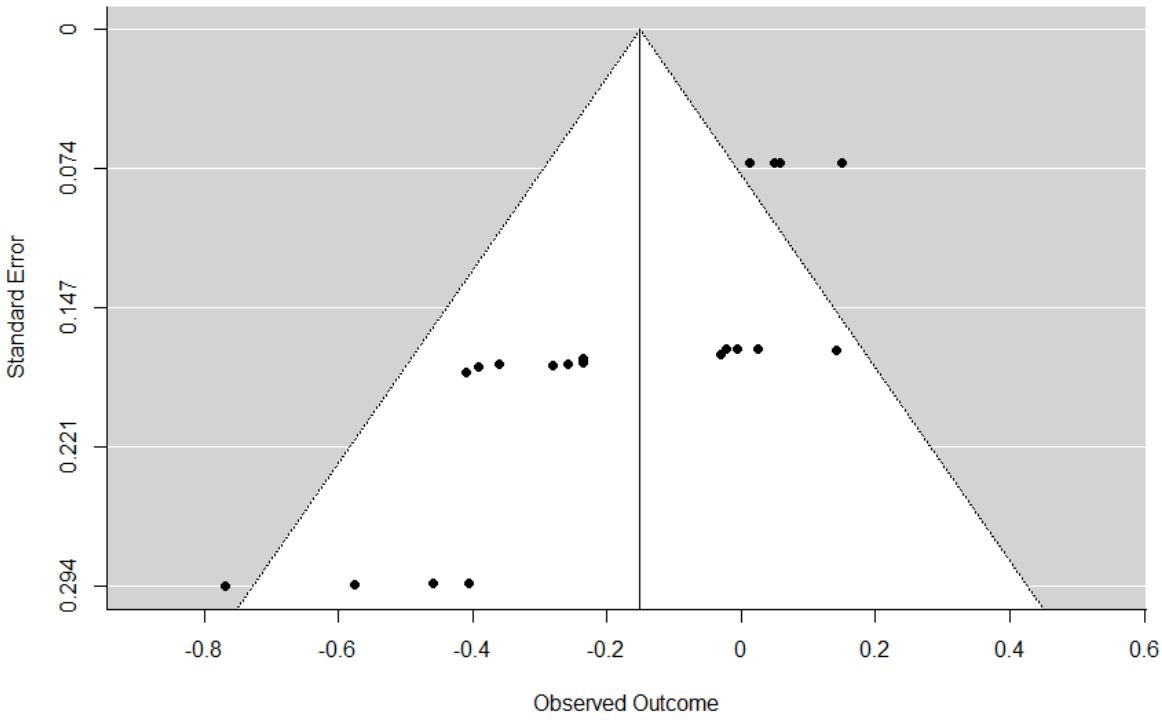
Supplementary Figure 24. Scatterplot of the association between age and effect size estimates in the meta-analysis investigating effects of a single dose of intranasal oxytocin on sensitivity to recognise emotions.



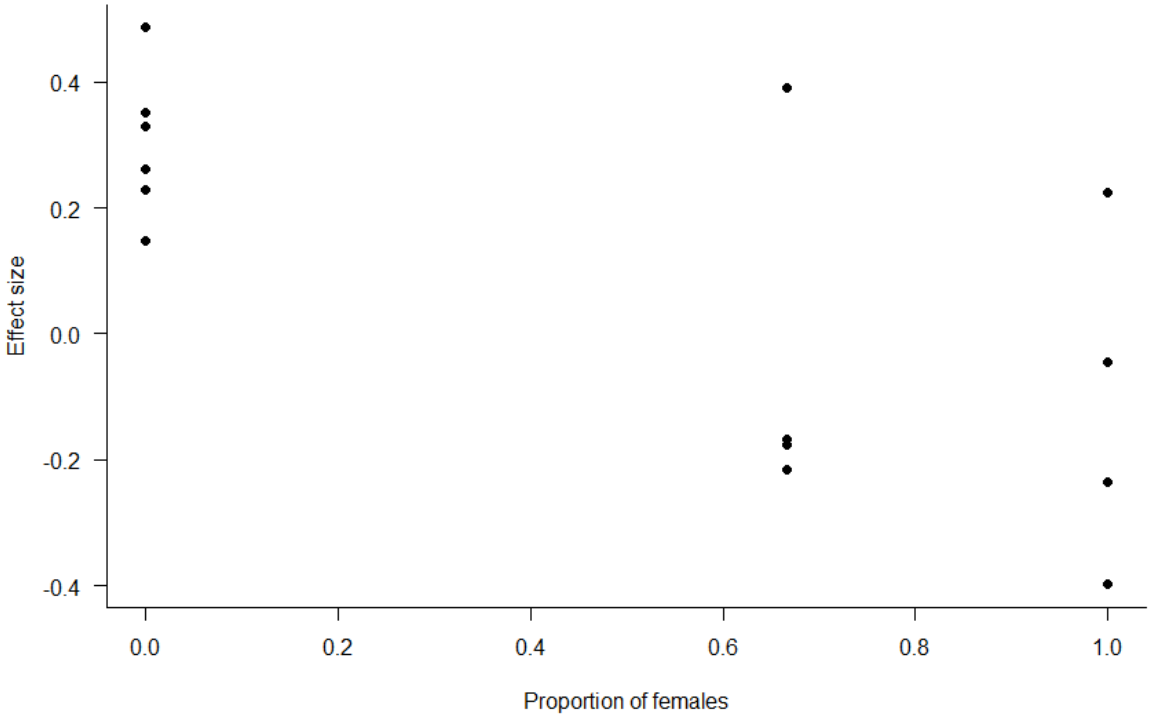
Supplementary Figure 25. Scatterplot of the association between dose (in IU) and effect size estimates in the meta-analysis investigating effects of a single dose of intranasal oxytocin on sensitivity to recognise emotions.



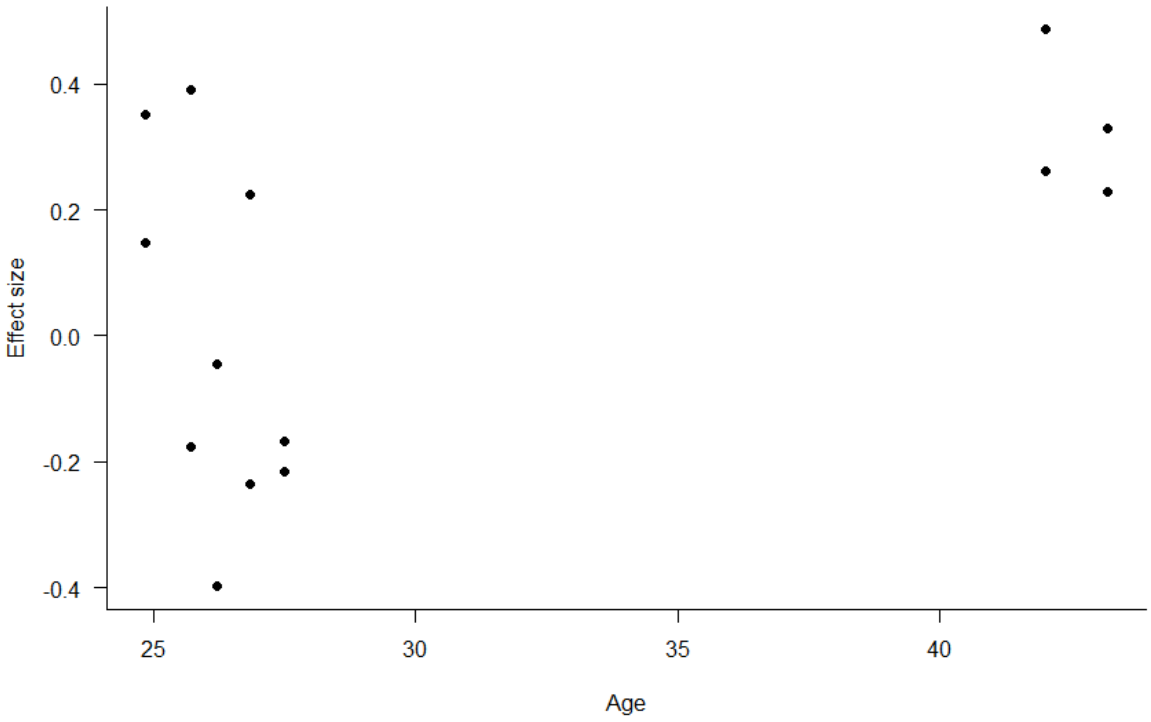
Supplementary Figure 26. Funnel plot of the meta-analysis investigating effects of a single dose of intranasal oxytocin on sensitivity to recognise emotions.



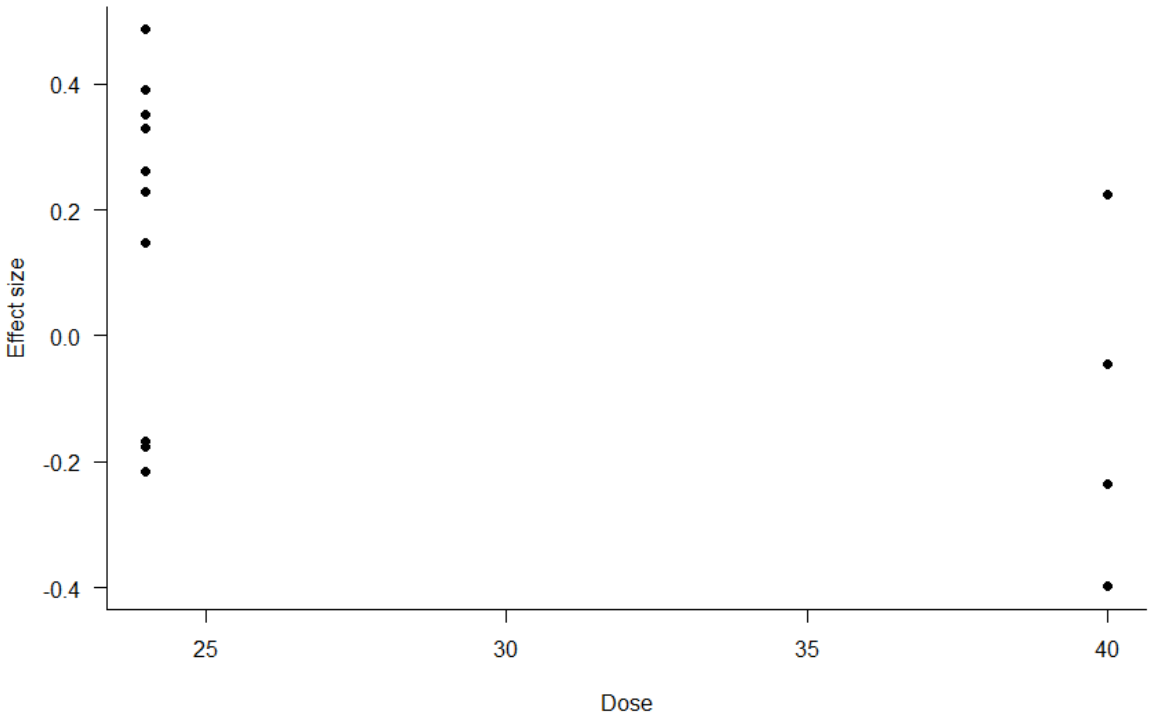
Supplementary Figure 27. Scatterplot of the association between proportion of female participants in the sample and effect size estimates in the meta-analysis investigating effects of a single dose of intranasal oxytocin on expression of emotions.



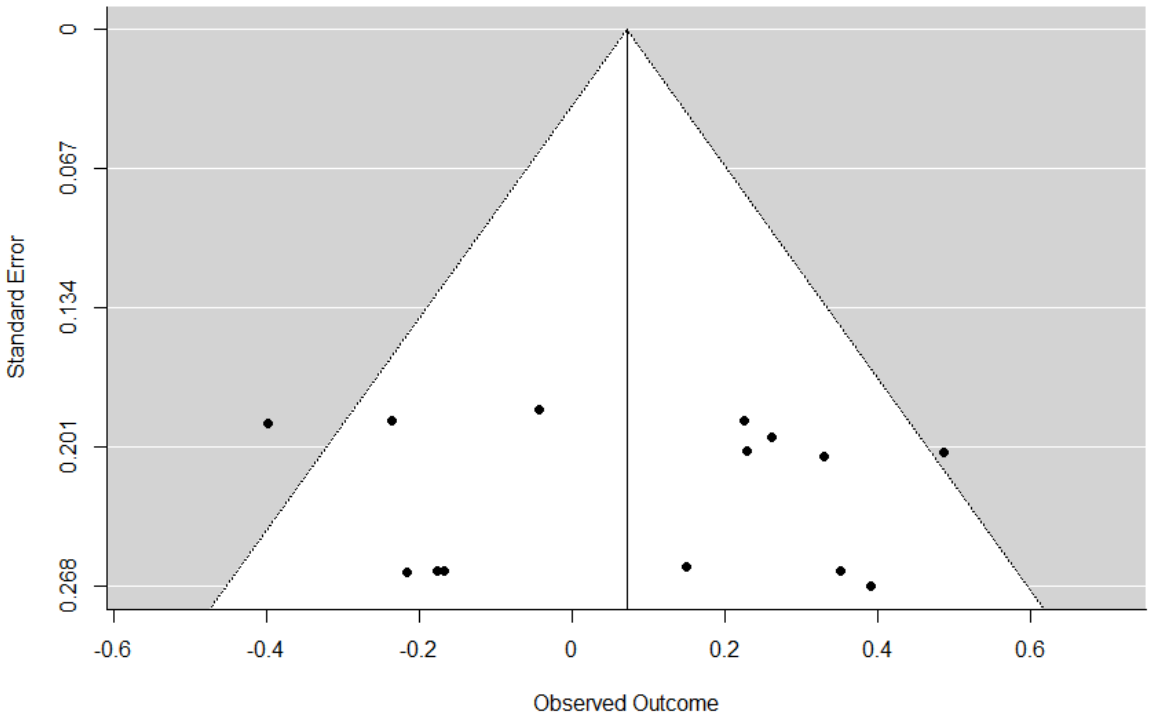
Supplementary Figure 28. Scatterplot of the association between age and effect size estimates in the meta-analysis investigating effects of a single dose of intranasal oxytocin on expression of emotions.



Supplementary Figure 29. Scatterplot of the association dose (in IU) and effect size estimates in the meta-analysis investigating effects of a single dose of intranasal oxytocin on expression of emotions.



Supplementary Figure 30. Funnel plot of the meta-analysis investigating effects of a single dose of intranasal oxytocin on expression of emotions.



CHAPTER 9:

9 Discussion

9.1 Summary of key findings

Table 1. Summary of key findings

Study	Aims	Findings
Chapter 2: The effects of negative and positive mood induction on eating behaviour: A meta-analysis of laboratory studies in the healthy population and eating and weight disorders	The aim of the review was to review studies investigating the effects of experimentally induced negative and positive mood on eating behaviour across eating and weight disorders.	Experimentally induced negative mood did not have consistent effects among healthy individuals, but significantly increased food intake among restraint eaters and people with binge eating disorder. Positive mood induction significantly increased food consumption among healthy individuals. Although the impact of positive mood on food consumption among people with eating disorders did not reach significance, there was some indication of increased intake in people with AN and reduced intake among people with BN.

Study	Aims	Findings
Chapter 3: Blunted neural response to implicit negative facial affect in anorexia nervosa	The aim of the study was to examine the neural processes that underlie anomalies in implicit processing of facial affect in AN.	The findings revealed significant differences between the groups in three of the regions of interest. While viewing fearful faces the AN group showed significantly reduced activation in the left amygdala and a small cluster in the left VLPFC compared to the healthy participants. In response to the happy facial expressions, the AN group showed significantly greater activation of the right posterior insula relative to the healthy participants. There were no significant group differences in activation of the bilateral fusiform gyrus in response to happy or fearful faces.
Chapter 4: FMRI Study of Neural Responses to Implicit Infant Emotion in Anorexia Nervosa	The aim of this study was to expand on the findings from Chapter 3 and explore differences in the neural processes involved in processing infant facial affect between people with AN and healthy individuals.	The findings showed significant groups differences in all three regions of interest. Participants with AN show significantly increased activation in the bilateral amygdala

Study	Aims	Findings
		and right DLPFC in response to positively valenced infant faces when compared to healthy participants. In response to negatively valenced infant faces, the AN participants showed significantly increased activation in the left posterior insula relative to the healthy participants.
Chapter 5: The effects of intranasal oxytocin on smoothie intake, cortisol and attentional bias in anorexia nervosa	The aim of this experimental study was to explore the potential effects of a single dose of intranasal oxytocin on smoothie intake as well as threat-related processes such as biological stress and attentional bias towards food images in AN.	Intranasal oxytocin significantly reduced salivary cortisol levels in the AN group but not in the healthy comparison participants. Oxytocin also significantly altered attentional bias towards food images among the AN participants only. However, this effect was only apparent in trials that were separated by very short inter-trial intervals and after participants had been exposed to the smoothie challenge, but not before. Single dose of intranasal oxytocin did not have a significant impact on self-reported anxiety or smoothie intake during

Study	Aims	Findings
		the standard laboratory challenge in the AN or healthy comparison groups.
Chapter 6: Effects of Intranasal Oxytocin on the Interpretation and Expression of Emotions in Anorexia Nervosa	The aim of this experimental study was to examine the effects of a single dose of intranasal oxytocin on interpretation and expression of emotions in people with AN.	intranasal oxytocin did not have a significant effect on interpretation of complex emotions or on spontaneous expression of emotions in response to emotionally provoking stimuli in the participants with AN or the healthy comparison individuals. As expected, the AN participants showed significantly less positive facial affect in response to the positive stimuli relative to the healthy comparison group. Unexpectedly, The AN group were also significantly more accurate when interpreting complex emotions than the healthy comparison group.
Chapter 7: Meta-analytic review of the effects of a single dose of	The aim of this review was to systematically review the evidence thus far on the effects of a single dose	The meta-analyses revealed that intranasal oxytocin increased physiological startle response to threatening

Study	Aims	Findings
intranasal oxytocin on threat processing in humans	of intranasal oxytocin on threat processing among healthy and clinical populations.	stimuli, but did not significantly impact attentional responses to threat in healthy or clinical groups. The systematic review exploring the effects of oxytocin on behavioural approach and avoidance responses to threat revealed mixed findings suggesting that little is currently known about the effects of oxytocin on these processes.
Chapter 8: Meta-analysis of the effects of intranasal oxytocin on interpretation and expression of emotions	The aim of this review was to synthesise studies investigating the effects of a single dose of intranasal oxytocin on interpretation and expression of emotions among healthy and clinical populations.	The meta-analytic review found that intranasal oxytocin significantly improved accuracy to recognise basic emotions, which was largely driven by significant improvement in recognition of fear and near significant improvement in recognition of disgust, among healthy populations only. There was also a significant oxytocin-induced improvement in the expression of positive emotions among healthy

Study	Aims	Findings
		individuals. However, this was not a robust finding, suggesting that substantial publication bias was present. There were no significant effects of oxytocin among the mixed clinical population.

9.2 Synthesis with existing literature

9.2.1 Impact of negative and positive mood on eating behaviour

Negative mood, elevated stress and anxiety have been found to precede episodes of binge eating and food restriction in people with eating disorders (Engel et al., 2013; Lavender et al., 2013). These findings have led to the suggestion that eating disorder behaviours may be compensatory mechanisms used to cope with negative emotions (Haynos and Fruzzetti, 2011; Brockmeyer et al., 2012). However, these findings are based on daily momentary assessments, which have been received criticism for relying on self-report and participants competence, and having no means of examining or confirming compliance (Smyth et al., 2001; Shiffman et al., 2008). Chapter 2 in this thesis added to the literature by synthesising experimental studies that examined the impact of induced negative and positive mood on eating behaviour. The findings from the experimental studies corroborate findings from the ecological momentary assessments suggesting that negative mood increases caloric consumption in people with binge eating forms of eating disorders as well as in people at risk of developing eating disorders. Additionally, these findings give further support to the notion that core eating disorder behaviours may be compensatory behaviours used to control negative mood (Haynos and Fruzzetti, 2011; Brockmeyer et al., 2012).

Chapter 2 also revealed that fewer studies thus far have investigated the effects of experimentally induced positive mood on caloric consumption, particularly among people with eating disorders. Among healthy individuals, the effects of experimentally induced positive mood on eating behaviour were clearer with a significant increase in food intake following induction. This findings supports the notion that healthy individuals tend to engage in hedonic eating when experiencing joy or happiness in naturalistic settings (Macht, 1999). Among healthy populations, increased positive mood has also been found to elevate the pleasantness of food and increase consumption (Macht et al., 2002; Macht,

2008). The meta-analysis also showed that the few studies that included people with eating disorders found non-significant, but encouraging results, suggesting that further exploration of interventions targeting mood may be of interest. Still, further research is needed to further examine the effects of positive mood in eating disorders.

9.2.2 Neural correlates of social-emotional processing in AN

A recent systematic review found that few studies thus far have investigated the neural processes that underlie difficulties in social-emotional processing in AN (McAdams and Smith, 2015). Four studies investigating neural responses to social behaviour reported anomalies in the recruitment of prefrontal cortical regions among those with AN relative to healthy individuals (McAdams and Krawczyk, 2011; Schulte-Rüther et al., 2012; McAdams and Krawczyk, 2013, 2014). Other studies in the review reported using a wide range of different experimental paradigms and only two studies investigated neural responses to facial affect (McAdams and Smith, 2015). A study by Fonville et al. (2014) found increased activation of the fusiform gyrus in people with AN while viewing positive facial expressions of increasing intensity. Cowdrey et al. (2012), on the other hand, found no significant differences in amygdala activation between healthy individuals and people who had recovered from AN while viewing positive and negative facial expressions. Due to the relative paucity of studies investigating the neural processes that underlie implicit processing of social-emotional cues in AN, further research is needed.

Anomalies in processing facial affect have recently been found to extend across the life span and not be purely associated with elevated sensitivity to social rank and implicit social threat in AN (Troop and Baker, 2008; Cardi et al., 2013; Cardi et al., 2014a; Troop, 2016). A series of behavioural experiments by Cardi et al. (2014b) found that people with eating disorders show anomalies in reactivity to infant

emotional display. However, to our knowledge, no studies to date have examined neural processes that underlie processing of infant emotion in AN. Previous work among people with mood and anxiety disorders, common comorbid disorders in AN, has demonstrated anomalies in neural responses to infant emotion (Baeken et al., 2010; Schechter et al., 2012; Wonch et al., 2016). Relative to healthy individuals, people with depression and PTSD show elevated emotional reactivity in regions such as the amygdala and insula, as well as increased recruitment of regions associated with emotion regulation, including the PFC, in response to infant emotion (Baeken et al., 2010; Schechter et al., 2012; Wonch et al., 2016). These findings indicate atypical reactions to infant emotional display in these disorder, which could have potentially disruptive impact on the infant as demonstrated in the still-face paradigm (Weinberg and Tronick, 1994). As longitudinal studies have shown that children of mothers with AN have higher risk to develop emotional and conduct disorders (Micali et al., 2014), further exploration of neural processes that underlie atypical reactivity to infant emotion in AN is of interest.

Chapters 3 and 4 in this thesis have expanded the field by further investigating the neural correlates of implicit processing of adult and infant emotional facial expressions in AN. The findings revealed that the AN participants showed increased activation of the amygdala and prefrontal regions in response positively valenced infant stimuli. Similar pattern on activation was seen in the healthy comparison participants in response to fearful adult faces, while the AN participants showed a blunted response in these regions. A wealth of neuroimaging studies among healthy individuals has documented increased activation of prefrontal and amygdala regions while viewing negative and threat-related cues and reduced recruitment of these regions in response to positive social-emotional cues (Fitzgerald et al., 2006; Marumo et al., 2009; Hung et al., 2010; Gold et al., 2015; Yanagisawa et al., 2016). Conversely, people with AN show reduced activation of lateral and medial PFC regions and the amygdala while viewing negative social-emotional stimuli and social behaviour (McAdams and Smith,

2015; Bang et al., 2016). Furthermore, people with mood disorders show similarly increased prefrontal and amygdala activation in response to positive social-emotional cues, such as smiling faces and positive infant stimuli (Grimm et al., 2008; Jaworska et al., 2015; Wonch et al., 2016). Taken together, it is possible that people with AN may react to positive infant stimuli as though it is negative or threatening, while failing to respond appropriately to negative adult social-emotional cues.

Relative to the healthy comparison participants, the AN group also had increased activation in the posterior insula in response to happy adult faces and negatively valenced infant faces. Among healthy individuals, increased activation of the bilateral posterior insula has been reported in response to subjective pain, negative emotional experiences, and active up-regulation of negative emotions (Craig, 2009; Singer et al., 2009; Duerden et al., 2013; Grecucci et al., 2013; Waugh et al., 2016). Posterior insula has also been implicated in distress and intense sadness in depression and PTSD (Mayberg et al., 1999; Schechter et al., 2012). Interestingly, people scoring high on measures of alexithymia also show increased activation of the posterior insula in response to emotionally provoking stimuli (Kano and Fukudo, 2013). These findings have been suggested to indicate atypical elevated distress and sympathetic arousal (Schechter et al., 2012; Kano and Fukudo, 2013). Taken together, people with AN may have specific difficulties in atypical reactivity to salient adult and infant social-emotional cues possibly suggesting elevated subjective distress and alexithymia.

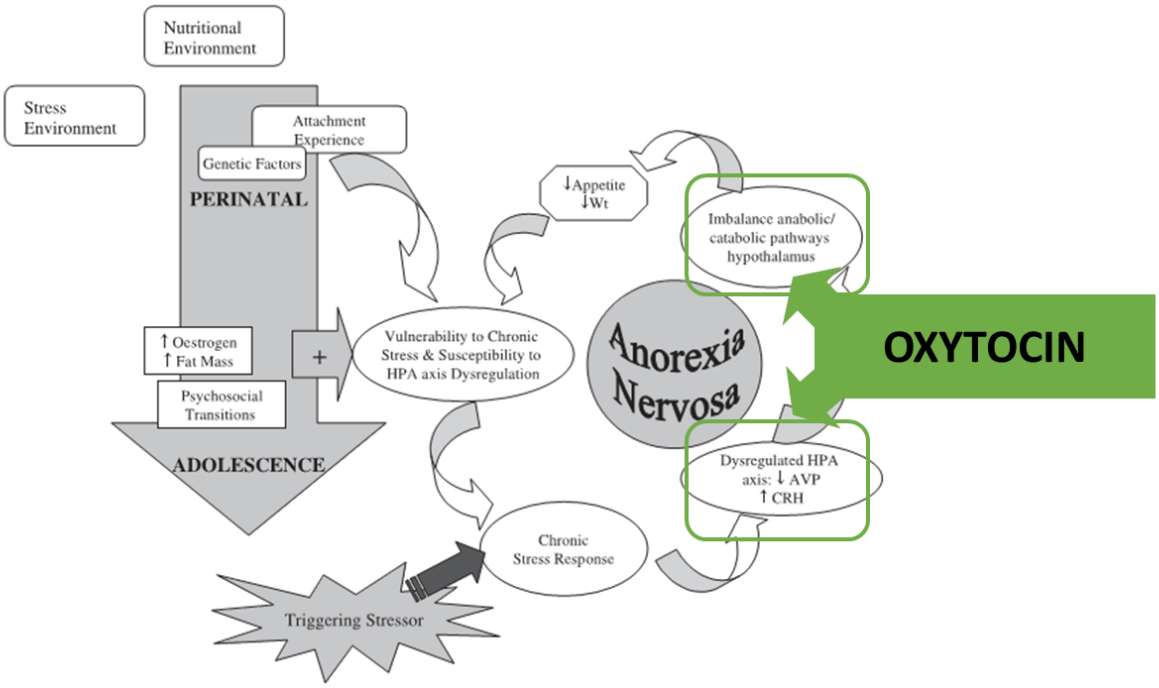
9.2.3 The potential of intranasal oxytocin as a new treatment

Preclinical studies have suggested that the neuropeptide oxytocin plays a role in several distinct functions, including stress and threat processing, and social functioning (Onaka, 2004; Yoshida et al., 2009; Onaka et al., 2012). These findings have sparked a great deal of interest in examining the effect of intranasally delivered oxytocin among humans, particularly among those with disorders

characterised by anomalies in the above mechanisms. A recent meta-analytic review found that a single dose of intranasal oxytocin led to a significant reduction in cortisol response to stress among clinical populations characterised by hyperactivation and dysregulation of the HPA axis (Cardoso et al., 2014). Along the same lines, several recent experimental studies have found that oxytocin may influence anxiety- and threat-related behaviours in humans, for instance reducing attention towards threatening stimuli (Bertsch et al., 2013; Domes et al., 2013b; Domes et al., 2013a; Kim et al., 2014a). Furthermore, previous systematic reviews have reported that oxytocin has a positive impact on social-emotional processing, increasing trust and cooperation, and improving recognition of facial affect (Van Ijzendoorn and Bakermans-Kranenburg, 2012; Shahrestani et al., 2013). Taken together, these findings indicate that oxytocin may be a promising treatment for psychiatric disorders.

Chapters 5 – 8 built on the existing literature by further investigating the effects of a single dose of intranasal oxytocin on social-emotional processing, stress and threat sensitivity. The findings revealed the intranasal oxytocin may be effective targeting physiological stress among people with AN (**Figure 1**). These findings are in line with previous work investigating the effects of oxytocin on stress response among clinical populations (Russell et al., 2013; Cardoso et al., 2014). A recent meta-analytic review found that intranasal oxytocin significantly reduced peripheral cortisol response to laboratory stress paradigms with a moderate effect size among clinical populations, including people with depression, BPD, substance dependence disorder, and fragile X syndrome (Cardoso et al., 2014). More to the point, a recent 6-week pilot trial explored the effects of daily repeat-administration of intranasal oxytocin on stress and anxiety among inpatients with AN (Russell et al., 2013). The authors found that over time oxytocin reduced daily salivary cortisol levels and cortisol response to an afternoon snack, which consisted of highly palatable food (Russell et al., 2013).

Figure 1. Likely site of oxytocin action on physiological stress in AN.

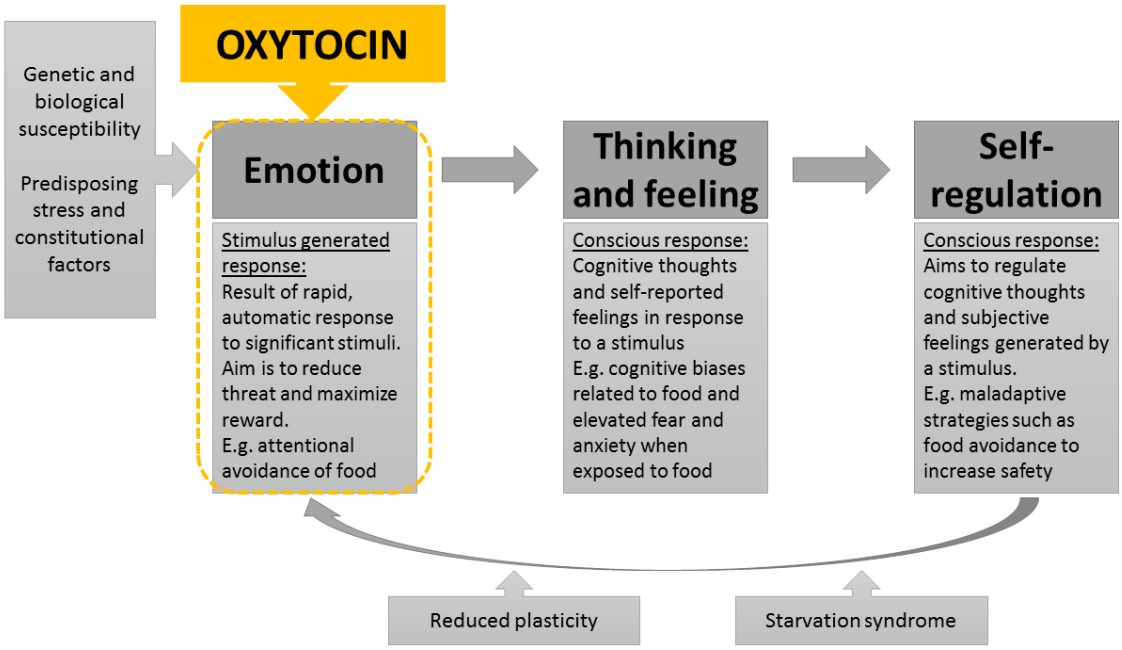


Despite having a strong effect on physiological stress, to our knowledge few studies to date have found significant effects of oxytocin on subjective anxiety or mood (Alvares et al., 2012; Churchland and Winkielman, 2012). Additionally, considering the close relationship between mood and eating behaviour, it is unsurprising that the effects of intranasal oxytocin on eating behaviour in eating disorders have been mixed. Chapter 5 in this thesis did not find any significant effects of a single dose of intranasal oxytocin on anxiety or smoothie intake during a standard laboratory challenge among participants with AN or healthy individuals. Similarly, the pilot 6-week trial by Russell et al. (2013) also did not find significant improvements in self-reported anxiety or changes in eating behaviour among inpatients with AN. Furthermore, a previous experimental study also failed to find significant changes in juice consumption in the laboratory or food intake during the subsequent 24 hours in people with AN (Kim et al., 2014a; Kim et al., 2015). However, the authors found that oxytocin significantly reduced food intake during the subsequent 24 hours among people with bulimia nervosa (Kim et al., 2015).

These findings raise some questions about the efficacy and effectiveness of intranasal oxytocin in the treatment of AN and suggest that engagement in eating disorder behaviours may not be directly connected to physiological stress.

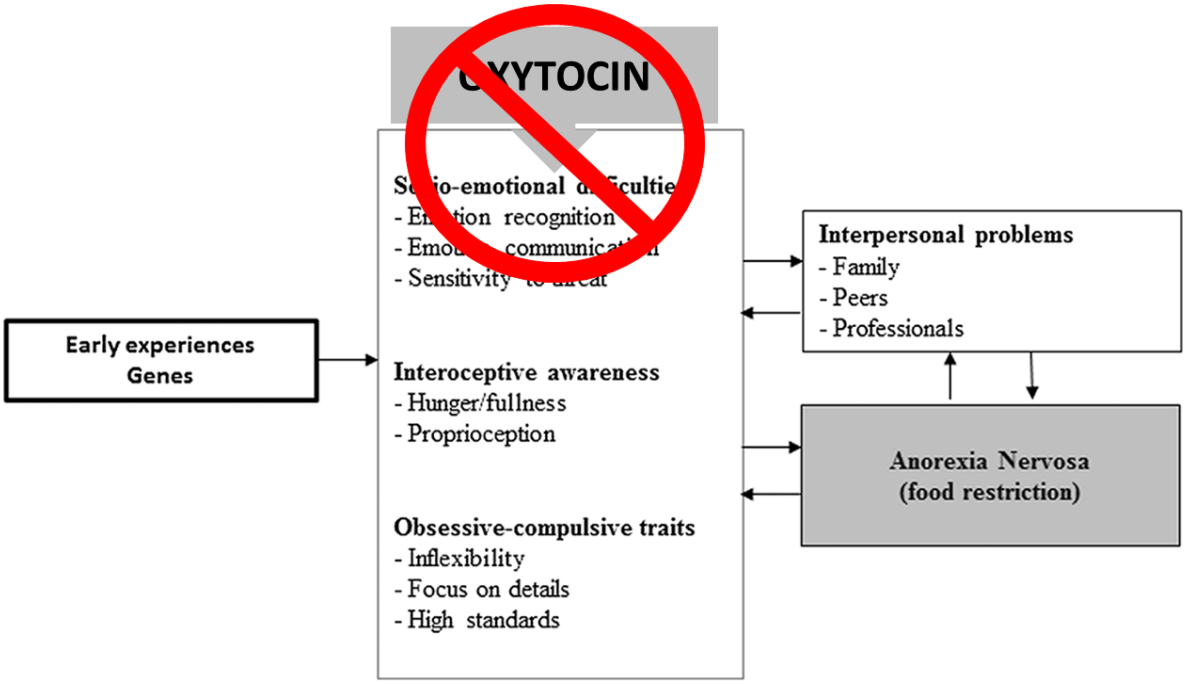
Experimental work conducted as part of this thesis showed that intranasal oxytocin had some effects on threat-related processing in AN (**Figure 2**). Oxytocin appeared to modulate attentional bias towards food-related images in people with AN, but it's effects were considerably dependent on task specific variables and the context in which the task was delivered. Similarly, in our meta-analytic review examining the effects of intranasal oxytocin on threat processing in general across healthy and clinical populations, we found that its effects on attentional responses were modulated by task specific and individual differences. The review also revealed that in some paradigms intranasal oxytocin led to an increase in the startle response to threat among healthy people. Contextual factors and individual differences have been previously suggested to be important factors influencing the effects of intranasal oxytocin in humans (Bartz et al., 2011; Olff et al., 2013). Indeed, other behavioural studies have found that in some contexts oxytocin may facilitate positive responding and reduce anxiety, while in others the effects of oxytocin may be counterproductive promoting fear and distrust (Shamay-Tsoory et al., 2009; Declerck et al., 2014; Daughters et al., 2017). Importantly, the present findings, along with previous work, show that oxytocin-induced alterations in automated attentional responses to food images in AN, do not seem to translate to improvements in self-reported mood or eating behaviour (Kim et al., 2014a). Although further exploration of the effects of oxytocin on threat-related processes is of interest, these findings raise some questions about the therapeutic potential of intranasal oxytocin.

Figure 2. Potential site of oxytocin action on some threat-related processes in AN



Finally, the findings in this thesis demonstrate that intranasal oxytocin does not appear to have substantial effects on social-emotional processing in the AN or HC group (**Figure 3**). Our second meta-analytic review found that intranasal oxytocin did not significantly improve theory of mind, sensitivity to recognise emotions, or emotion expression. Oxytocin only improved basic emotion recognition accuracy with a small effect size, which was largely driven by improved recognition of fear and disgust, among healthy individuals. Taken together, these findings lend some support for the hypothesis that effects of oxytocin may be moderated by social boundaries (Olff et al., 2013; Zik and Roberts, 2015). The hypothesis suggests that oxytocin facilitates cooperation and trust between "in-group" members, who are deemed "safe", while increasing distrust and defensiveness towards members of an "out-group", who are deemed "unsafe" (Olff et al., 2013; Zik and Roberts, 2015). Under laboratory conditions this may be seen as improved recognition of negative social cues in unfamiliar faces and increased alertness to potential threats. Thus, effects of oxytocin may be protective, at least among healthy individuals, but further research is still needed.

Figure 3. Oxytocin does not appear to impact social-emotional processing in AN.



9.3 Implications and future directions

Findings from the studies in this thesis have implications to understanding the aetiology of AN as well as on the treatment of AN. A number of theoretical models of AN highlight the role of elevated negative mood, anxiety, and threat sensitivity as important predisposing and maintenance factors (Connan et al., 2003; Hatch et al., 2010; Treasure and Schmidt, 2013; Treasure and Cardi, 2017). The models suggest that prior to the onset of full illness people with AN show elevated sensitivity to stress and threat, and anxious-avoidant interpersonal style. These traits are believed to exacerbate interpersonal difficulties and increase social isolation. Together, these processes are thought to give salience to eating disorders thoughts fuelling core eating disorder behaviours, and give a false sense of safety and control.

The present findings give further support to these models, demonstrating that people with AN show anomalies in the negative valence system, including physiological stress, self-reported anxiety, attentional bias towards food stimuli, and food avoidance in a standard laboratory challenge. Although theoretical models and studies using ecological momentary assessments suggest that these processes may be closely related, the present findings indicate that this may not be the case. The present finding showed that although oxytocin reduced salivary cortisol and influenced attentional response, there was no significant change in self-reported anxiety or smoothie intake. Similarly, a longer pilot trial found that although repeated administration of oxytocin reduced salivary cortisol, there was no significant change in subjective anxiety or eating behaviour in inpatients with AN (Russell et al., 2013). These findings suggest that there may be a general disconnect between physiological state and subjective experience and behaviour in AN. This notion is supported by findings that people with AN show high alexithymia and have difficulties detecting and interpreting physiological signals of hunger (Pollatos et al., 2008; Kaye et al., 2013; Westwood et al., 2017). Thus, it may be that targeting physiological stress may not be sufficient in AN. It may be that the subjective experience of stress and

anxiety may be more important and further exploration of subjective experience and mood as potential targets for interventions may be of interest.

The present findings also document anomalies in social-emotional processing, including expression of emotions and implicit processing of facial affect, in people with AN. Two studies in this thesis further probed the neural processes that underlie these anomalies and the findings point towards atypical implicit responses to both adult and infant facial affect in AN. Atypical reactions to emotional display has been suggested to have negative emotional and social consequences (Gross, 2002; Butler et al., 2009; Szczurek et al., 2012). Experimental work has demonstrated that reduced response to negative emotional stimuli can increase blood pressure and subjective negative feelings, while expressing negative emotions can help reduce blood pressure (Butler et al., 2009). Another recent experimental study demonstrated that atypical blunted or inappropriate responses to emotional stimuli was met with negative social judgements and avoidance by others (Szczurek et al., 2012). Furthermore, atypical reactions to infant emotional display can have profoundly negative impact on the infant (Feldman, 2007, 2012). A good example of this is the famous still-face paradigm, which demonstrated the distressing effect of blunted facial response has on an infant (Weinberg and Tronick, 1994). Thus, interventions targeting anomalies in social-emotional processing in AN are of importance.

Finally, the findings presented here highlight the importance of further exploring the impact of contextual factors and individual differences on the effects of intranasal oxytocin in healthy and clinical populations. Particularly if intranasal oxytocin is to be considered as a potential treatment in psychiatric disorders in general, it is of importance to establish how certain contextual cues can cause the effects of oxytocin to become counterproductive. Furthermore, some experimental work has already documented that individual differences in early experiences can influence the effects of

oxytocin, with those without attachment difficulties showing more positive effects of oxytocin (Bakermans-Kranenburg et al., 2011; Fang et al., 2014). Furthermore, people who have experienced childhood trauma have been documented to have reduced levels of CSF oxytocin and anomalies in oxytocin response to stress (Heim et al., 2008; Pierrehumbert et al., 2010). Considering many individuals with AN or other psychiatric disorders report high incidence of insecure attachment and difficult early experiences (Marganska et al., 2013; Caglar-Nazali et al., 2014; Gumley et al., 2014; Manning et al., 2017), establishing the kinds of individual factors that could lead oxytocin to produce counterproductive effects is important. Thus, future research may benefit from further exploring the impact of these factors on the effects of oxytocin.

9.4 General limitations

The studies in this thesis are not without limitations. Study-specific limitations are detailed in the relevant chapters and some general limitations are summarised below.

The main limitation of the experimental studies in this thesis was the relatively small sample sizes used and heterogeneity of the samples. Small, heterogeneous sample size limits statistical power to detect a true effect and can lead to false negative findings or inflated effects and false positives (Zhang et al., 2013; Walum et al., 2016). The small sample sizes also prevented further investigation of potential sources of heterogeneity, such as AN subtypes, effect of anti-depressant medication on neural responses to social-emotional stimuli, and the differences between AN participants receiving treatment and those in the community. Future studies may benefit from exploring the impact of these factors and including a larger of participants to combat the impact of heterogeneity. Therefore, it is of importance that the findings from the experimental studies reported here are replicated before firm conclusions are drawn.

Another limitation is some of the tasks used in the experimental studies. For instance, although the gender identification task worked well with adult faces in Chapter 3, the participants in Chapter 4 were unable to accurately identify the gender of the infant faces. The participants in Chapter 4 were informed that the task was difficult and the main point was to attend to the stimuli and not spend too much time on the gender identification task. Still it is possible that such a difficult task could have impacted the results. Additionally, the difference in task performance was the primary reason we were not able to statistically compare neural responses to positively valenced adult and infant faces in the AN or healthy groups. These limitations highlight the need for further replication with different paradigms, before firm conclusions about the findings in this thesis can be drawn.

The tasks used to assess the impact of oxytocin on the negative valence system and the systems of social processes are also not without limitations. The dot-probe task, used to assess attentional bias, has been found to have poor internal validity raising some questions about any attentional bias scores derived from the reaction time data (Schmukle, 2005; Staugaard, 2009). The Reading the Mind in the Eyes task, used to assess recognition of complex emotions, focuses on the eye regions cropping out the rest of the face, creating non-naturalistic stimuli. Additionally, good performance on the Reading the Mind in the Eyes task also requires participants to be very proficient in English and pay great attention to detail. As people with AN show detail focus at the cost of the bigger picture (Lang et al., 2014), this could go some way to explain the findings presented here. Finally, the videos used to examine spontaneous production of facial affect were quite old and may have memories attached to them particularly in relation to family time. Such memories have been found to influence behaviour and experiences in adulthood (Maccari et al., 2014; Patchev et al., 2014), and it is possible that the present findings were confounded by subjective memories. Taken together, future studies would benefit from adopting different paradigms to assess the impact of oxytocin on attentional bias and social-emotional processing.

Another limitation of the experimental study investigating the effects of a single dose of intranasal oxytocin on salivary cortisol was that half of the AN participants were inpatients. The inpatients were assessed in the inpatient ward and they were due to have their meal shortly after the experimental session, which could have impacted the findings. Indeed, people with AN report elevated stress and anxiety around mealtimes (Cowdrey et al., 2013; Levinson and Byrne, 2015). This could have had an impact on the salivary cortisol levels, going at least some way to explain the within group heterogeneity. This along with the fact that the present study used a smoothie challenge, could explain why salivary cortisol levels appeared to increase in the placebo session. In fact, the oxytocin-induced

reduction in salivary cortisol observed in this study was not a significant reduction from baseline, but instead a significant reduction compared to what happened in the placebo condition. Future studies may benefit from further controlling for such confounding factors.

It is also possible that the effects of intranasal oxytocin on the various tasks was influenced by individual differences, such as alexithymia or attachment style. Previous work has found that these factors influence the effects of intranasal oxytocin on tasks assessing various aspects of social cognition (Bakermans-Kranenburg et al., 2011; Luminet et al., 2011; Fang et al., 2014). Furthermore, recent theoretical models have suggested that the effects of oxytocin could also be influenced by social boundaries, the formation of which is likely impacted by individual differences in attachment style and previous experiences (Olff et al., 2013; Zik and Roberts, 2015). As such individual differences were not assessed in the studies assessing the impact of oxytocin, it is not possible to determine whether they had an impact here. Therefore, future studies may benefit from further exploring the influence individual differences have on the effects of oxytocin.

The main limitation of the systematic reviews in this thesis was the heterogeneity between different studies. The studies included in Chapter 2 included a wide range of different outcome measures, ranging from calories to number of cookies consumed during the test meal, and a number of different procedures were used to induce positive and negative mood. In the studies included in Chapters 7 and 8 several different doses of intranasal oxytocin and different tasks were used to assess interpretation and expression of emotions as well as threat processing. These factors may have had some impact on the results and examining their impact in meta-regressions was not always possible. Therefore, future research may benefit from further examining the impact of procedural differences in experimental

research to gain better understating of the effects of mood on eating behaviour as well as the effects of intranasal oxytocin in humans.

The systematic reviews also included few studies with clinical groups. Indeed, the conclusion from most of the reviews was that there was a relative paucity of research examining the impact of experimentally induced positive and negative mood on eating behaviour, the impact of a single dose of intranasal oxytocin on interpretation and expression of emotions, the impact of a single dose of intranasal oxytocin on threat processing among clinical populations. This makes it difficult to draw conclusions at this stage and more research with large sample sizes is still needed.

Finally, all studies included in this thesis focused on examining cross-sectional differences. Cross-sectional studies allow examination of multiple outcomes and generation of further hypotheses, but they are not without limitations. In cross-sectional studies it is difficult to determine whether differences observed between the people with acute AN and a healthy comparison group are due to state of malnutrition. Additionally, it is difficult to determine long-term effects of interventions as well as the impact of medication and stage of illness. Thus, further longitudinal research is needed.

9.5 Overall conclusion

This thesis aimed to examine anomalies in the negative valence system and in the systems of social processes in AN as well as explore the question whether oxytocin is useful candidate to target these processes. The findings revealed that negative mood and stress are likely to contribute to core eating disorders behaviours. Additionally, while a single dose of intranasal oxytocin reduced salivary cortisol and altered attentional responses to food images, it did not significantly improve self-reported anxiety or eating behaviour during a standard laboratory test meal. In a further meta-analytic review, we also found that across healthy and clinical populations a single dose of intranasal oxytocin has small and complex effects on threat-related processing in general, which are strongly influenced by individual and contextual factors. The findings in this thesis also showed that people with AN show neurofunctional anomalies during implicit processing of both adult and infant facial affect. A single dose of intranasal oxytocin did not significantly alter these anomalies social-emotional processing in AN. Furthermore, our series of meta-analyses showed that a single dose of intranasal oxytocin does not have substantial effects on social-emotional processing in general among healthy or clinical populations. Although further research is still needed, taken together, these findings suggest that intranasal oxytocin may not be a useful in the treatment of AN. The findings in this thesis further suggest that although physiological stress is likely to contribute to core eating disorder behaviours, interventions focusing solely on reducing stress hormone levels are unlikely to produce substantial changes in eating behaviour. Effective interventions targeting subjective experience of stress and anxiety as well as difficulties in social-emotional functioning are needed in the treatment of AN.

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Appendices

10.1 Eating Disorders Examination Questionnaire (EDEQ)

EDE-Q

The following questions are concerned with the past four weeks only (28 days). Please read each question carefully and tick the appropriate box.

On how many days out of the past 28 days...

	No days	1-5 days	6-12 days	13-15 days	16-22 days	23-27 days	Every day
Have you been deliberately trying to limit the amount of food you eat to influence your shape or weight?	◯	◯	◯	◯	◯	◯	◯
Have you gone for long periods of time (8 hours or more) without eating anything in order to influence your shape or weight?	◯	◯	◯	◯	◯	◯	◯
Have you tried to avoid eating foods which you like in order to influence your shape or weight?	◯	◯	◯	◯	◯	◯	◯
Have you tried to follow definite rules regarding your eating in order to influence your shape or weight; for example, a calorie limit, a set amount of food, or rules about what or when you should eat?	◯	◯	◯	◯	◯	◯	◯
Have you wanted your stomach to be empty?	◯	◯	◯	◯	◯	◯	◯
Has thinking about food or its calorie content made it much more difficult to concentrate on things you're interested in; for example, read, watch TV or follow a conversation?	◯	◯	◯	◯	◯	◯	◯
Have you been afraid of losing control over eating?	◯	◯	◯	◯	◯	◯	◯
Have you had episodes of binge eating?	◯	◯	◯	◯	◯	◯	◯
Have you eaten in secret? (Do not count binges)	◯	◯	◯	◯	◯	◯	◯
Have you definitely wanted your stomach to be flat?	◯	◯	◯	◯	◯	◯	◯
Has thinking about shape or weight made it more difficult to concentrate on things you are interested in; e.g., read, watch TV or follow a conversation?	◯	◯	◯	◯	◯	◯	◯
Have you had a definite fear that you might gain weight or become fat?	◯	◯	◯	◯	◯	◯	◯
Have you felt fat?	◯	◯	◯	◯	◯	◯	◯
Have you had a strong desire to lose weight?	◯	◯	◯	◯	◯	◯	◯

Over the past 4 weeks (28 days)...

	None of the times	A few of the times	Less than half the time	Half the time	More than half the time	Most of the time	Every time
On what proportion of times that you have eaten have you felt guilty because of the effect on your shape or weight? (Do not count binges)	◯	◯	◯	◯	◯	◯	◯

Have there been any times over the past 4 weeks (28 days) when you have felt that you have eaten what other people would regard as an unusually large amount of food given the circumstances?

◯ No

◯ Yes

If yes, how many such episodes have you had over the past four weeks?

During how many of these episodes of overeating did you have a sense of having lost control over your eating?

Have you had other episodes of eating over the past 4 weeks (28 days) in which you have had a sense of having lost control and eaten too much, but have not eaten an unusually large amount of food given the circumstances?

◯ No

◯ Yes

If yes, how many such episodes have you had over the past four weeks?

Have you made yourself sick (vomit) over the past 4 weeks (28 days) as a means of controlling your shape or weight?

◯ No

◯ Yes

If yes, how many times have you done this over the past four weeks?

How many times have you done this over the past four weeks?

Have you taken laxatives over the past 4 weeks (28 days) as a means of controlling your shape or weight?

◯ No

◯ Yes

If yes, how many times have you done this over the past four weeks?

Oxytocin Survey

Have you taken diuretics (water tablets) over the past 4 weeks (28 days) as a means of controlling your shape or weight?

- ◯ No
- ◯ Yes

If yes, how many times have you done this over the past four weeks?

Have you exercised hard over the past 4 weeks (28 days) as a means of controlling your shape or weight?

- ◯ No
- ◯ Yes

How many times have you done this over the past four weeks?

Over the past 4 weeks (28 days)...

	0 (Not at all)	1	2 (slightly)	3	4 (moderately)	5	6 (markedly)
Has your weight influenced how you think about (judge) yourself as a person?	◯	◯	◯	◯	◯	◯	◯
Has your shape influenced how you think about (judge) yourself as a person?	◯	◯	◯	◯	◯	◯	◯
How much would it upset you if you had to weigh yourself once a week for the next four weeks?	◯	◯	◯	◯	◯	◯	◯
How dissatisfied have you felt about your weight?	◯	◯	◯	◯	◯	◯	◯
How dissatisfied have you felt about your shape?	◯	◯	◯	◯	◯	◯	◯
How concerned have you been about other people seeing you eat?	◯	◯	◯	◯	◯	◯	◯
How uncomfortable have you felt seeing your body; for example, in shop window reflections, while undressing or taking a bath or shower?	◯	◯	◯	◯	◯	◯	◯
How uncomfortable have you felt about others seeing your body; for example, in communal changing rooms, when swimming or wearing tight clothes?	◯	◯	◯	◯	◯	◯	◯

10.2 Depression, Anxiety, and Stress Scale (DASS)

DASS 21 - Depression, Anxiety and Stress Scale

Please read each statement and tick a number 0, 1, 2 or 3 which indicates how much the statement applied to you over the past week.
There are no right or wrong answers. Do not spend too much time on any statement.

0 - Did not apply to me at all
1 - Applied to me to some degree, or some of the time
2 - Applied to me to a considerable degree, or a good part of time
3 - Applied to me very much, or most of the time

	0	1	2	3
I found it hard to wind down	◯	◯	◯	◯
I was aware of dryness of my mouth	◯	◯	◯	◯
I couldn't seem to experience any positive feeling at all	◯	◯	◯	◯
I experienced breathing difficulty (e.g., excessively rapid breathing, breathlessness in the absence of physical exertion)	◯	◯	◯	◯
I found it difficult to work up the initiative to do things	◯	◯	◯	◯
I tended to over-react to situations	◯	◯	◯	◯
I experienced trembling (eg, in the hands)	◯	◯	◯	◯
I felt that I was using a lot of nervous energy	◯	◯	◯	◯
I was worried about situations in which I might panic and make a fool of myself	◯	◯	◯	◯
I felt that I had nothing to look forward to	◯	◯	◯	◯
I found myself getting agitated	◯	◯	◯	◯
I found it difficult to relax	◯	◯	◯	◯
I felt down-hearted and blue	◯	◯	◯	◯
I was intolerant of anything that kept me from getting on with what I was doing	◯	◯	◯	◯
I felt I was close to panic	◯	◯	◯	◯
I was unable to become enthusiastic about anything	◯	◯	◯	◯
I felt I wasn't worth much as a person	◯	◯	◯	◯
I felt that I was rather touchy	◯	◯	◯	◯
I was aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat)	◯	◯	◯	◯
I felt scared without any good reason	◯	◯	◯	◯
I felt that life was meaningless	◯	◯	◯	◯

10.3 Autism Spectrum Quotient (short version; AQ-10)

AQ-10

Please select one option per question only:

	Definitely agree	Slightly agree	Slightly disagree	Definitely disagree
I often notice small sounds when others do not	◯	◯	◯	◯
I usually concentrate more on the whole picture, rather than the small details	◯	◯	◯	◯
I find it easy to do more than one thing at once	◯	◯	◯	◯
If there is an interruption, I can switch back to what I was doing very quickly	◯	◯	◯	◯
I find it easy to 'read between the lines' when someone is talking to me	◯	◯	◯	◯
I know how to tell if someone listening to me is getting bored	◯	◯	◯	◯
When I'm reading a story I find it difficult to work out the characters' intentions	◯	◯	◯	◯
I like the collect information about categories of things (e.g. types of car, types of bird, types of train, types of plant, etc.)	◯	◯	◯	◯
I find it easy to work out what someone is thinking or feeling just by looking at their face	◯	◯	◯	◯
I find it difficult to work out people's intentions	◯	◯	◯	◯